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### INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: WO 98/49311 (11) International Publication Number: A2 C12N 15/29, 15/62, 15/70, 15/86, A61K (43) International Publication Date: 38/16, 48/00

5 November 1998 (05.11.98)

(21) International Application Number: PCT/CA98/00394

(22) International Filing Date: 30 April 1998 (30.04.98)

(30) Priority Data:

30 April 1997 (30.04.97) US 60/045,148 29 October 1997 (29.10.97) US 60/063,715

(71) Applicant (for all designated States except US): DE NOVO ENZYME CORPORATION [CA/CA]; #2 Suite SFU Discovery Park, Burnaby, British Columbia V5A 1S6 (CA).

(72) Inventor; and

(75) Inventor/Applicant (for US only): BORGFORD. Thor [CA/CA]; 443 Fadar Street, New Westminster, British Columbia V3L 3T2 (CA).

(74) Agent: BERESKIN & PARR; 40th floor, 40 King Street West, Toronto, Ontario M5H 3Y2 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

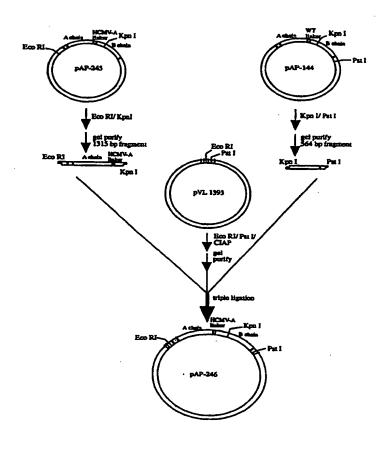
#### **Published**

Without international search report and to be republished upon receipt of that report.

(54) Title: RICIN-LIKE TOXIN VARIANTS FOR TREATMENT OF CANCER, VIRAL OR PARASITIC INFECTIONS

#### (57) Abstract

The present invention provides a protein having an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains. The linker sequence contains a cleavage recognition site for a disease specific protease such as a cancer, fungal, viral or parasitic protease. The invention also relates to a nucleic acid molecule encoding the protein and to expression vectors incorporating the nucleic acid molecule. Also provided is a method of inhibiting or destroying mammalian cancer cells, cells infected with a virus, a fungus, or parasite, or parasites utilizing the nucleic acid molecules and proteins of the invention and pharmaceutical compositions for treating human cancer, viral infection, fungal infection, or parasitic infection.



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### RICIN-LIKE TOXIN VARIANTS FOR TREATMENT OF CANCER, VIRAL OR PARASITIC INFECTIONS

### FIELD OF THE INVENTION

The invention relates to proteins useful as therapeutics against cancer, viral infections, parasitic and fungal infections. The proteins contain A and B chains of a ricin-like toxin linked by a linker sequence that is specifically cleaved and activated by proteases specific to disease-associated pathogens or cells.

### **BACKGROUND OF THE INVENTION**

Bacteria and plants are known to produce cytotoxic proteins which may consist of one, two or several polypeptides or subunits. Those proteins having a single subunit may be loosely classified as Type I proteins. Many of the cytotoxins which have evolved two subunit structures are referred to as type II proteins (Saelinger, C.B. in Trafficking of Bacterial Toxins (eds. Saelinger, C.B.) 1-13 (CRC Press Inc., Boca Raton, Florida, 1990). One subunit, the A chain, possesses the toxic activity whereas the second subunit, the B chain, binds cell surfaces and mediates entry of the toxin into a target cell. A subset of these toxins kill target cells by inhibiting protein biosynthesis. For example, bacterial toxins such as diphtheria toxin or Pseudomonas exotoxin inhibit protein synthesis by inactivating elongation factor 2. Plant toxins such as ricin, abrin, and bacterial toxin Shiga toxin, inhibit protein synthesis by directly inactivating the ribosomes (Olsnes, S. & Phil, A. in Molecular action of toxins and 25 viruses (eds. Cohen, P. & vanHeyningen, S.) 51-105 Elsevier Biomedical Press, Amsterdam, 1982).

Ricin, derived from the seeds of Ricinus communis (castor oil plant), may be the most potent of the plant toxins. It is estimated that a single ricin A chain is able to inactivate ribosomes at a

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rate of 1500 ribosomes/minute. Consequently, a single molecule of ricin is enough to kill a cell (Olsnes, S. & Phil, A. in Molecular action of toxins and viruses (eds. Cohen, P. & vanHeyningen, S.) (Elsevier Biomedical Press, Amsterdam, 1982). The ricin toxin is a glycosylated heterodimer consisting of A and B chains with molecular masses of 30,625 Da and 31,431 Da linked by a disulphide bond. The A chain of ricin has an N-glycosidase activity and catalyzes the excision of a specific adenine residue from the 28S rRNA of eukaryotic ribosomes (Endo, Y. & Tsurugi, K. J., Biol. Chem. 262:8128 (1987)). The B chain of ricin, although not toxic in itself, promotes the toxicity of the A chain by binding to galactose residues on the surface of eukaryotic cells and stimulating receptor-mediated endocytosis of the toxin molecule (Simmons et al., Biol. Chem. 261:7912 (1986)). Once the toxin molecule consisting of the A and B chains is internalized into the cell via clathrin-dependent or independent mechanisms, the greater reduction potential within the cell induces a release of the active A chain, eliciting its inhibitory effect on protein synthesis and its cytotoxicity (Emmanuel, F. et al., Anal. Biochem. 173: 134-141 (1988); Blum, J.S. et al., J. Biol. Chem. 266: 22091-22095 (1991); Fiani, M.L. et al., Arch. Biochem. Biophys. 307: 225-230 (1993)). Empirical evidence suggests that activated toxin (e.g. ricin, shiga toxin and others) in the endosomes is transcytosed through the trans-Golgi network to the endoplasmic reticulum by retrograde transport before the A chain is translocated into the cytoplasm to elicit its action (Sandvig, K. & van Deurs, B., FEBS Lett. 346: 99-102 (1994).

Protein toxins are initially produced in an inactive, precursor form. Ricin is initially produced as a single polypeptide (preproricin) with a 35 amino acid N-terminal presequence and 12 amino acid linker between the A and B chains. The pre-sequence is removed during translocation of the ricin precursor into the endoplasmic reticulum (Lord, J.M., Eur. J. Biochem. 146:403-409 (1985) and Lord, J.M., Eur. J. Biochem. 146:411-416 (1985)). The proricin is then

translocated into specialized organelles called protein bodies where a plant protease cleaves the protein at a linker region between the A and B chains (Lord, J.M. et al., FASAB Journal 8:201-208 (1994)). The two chains, however, remain covalently attached by an interchain disulfide bond (cysteine 259 in the A chain to cysteine 4 in the B chain) and mature disulfide linked ricin is stored in protein bodies inside the plant cells. The A chain is inactive in proricin (O'Hare, M. et al., FEBS Lett. 273:200-204 (1990)) and it is inactive in the disulfide-linked mature ricin (Richardson, P.T. et al., FEBS Lett. 255:15-20 (1989)). The ribosomes of the castor bean plant are themselves susceptible to inactivation by ricin A chain; however, as there is no cell surface galactose to permit B chain recognition the A chain cannot re-enter the cell. The exact mechanism of A chain release and activation in target cell cytoplasm is not known (Lord, J.M. et al., FASAB Journal 8:201-208 (1994)). However, it is known that for activation to take place the disulfide bond between the A and B chains must be reduced and, hence, the linkage between subunits broken.

Diphtheria toxin is produced by Corynebacterium diphtheriae as a 535 amino acid polypeptide with a molecular weight of approximately 58kD (Greenfield, L. et al., Proc. Natl. Acad. Sci. USA 20 80:6853-6857 (1983); Pastan, I. et al., Annu. Rev. Biochem. 61:331-354 (1992); Collier, R.J. & Kandel, J., J. Biol. Chem. 246:1496-1503 (1971)). It is secreted as a single-chain polypeptide consisting of 2 functional Similar to proricin, the N-terminal domain (A-chain) contains the cytotoxic moiety whereas the C-terminal domain (B-chain) is responsible for binding to the cells and facilitates toxin endocytosis. Conversely, the mechanism of cytotoxicity for diphtheria toxin is based on ADP-ribosylation of EF-2 thereby blocking protein synthesis and producing cell death. The 2 functional domains in diphtheria toxin are linked by an arginine-rich peptide sequence as well as a disulphide bond. Once the diphtheria toxin is internalized into the cell, the arginine-rich peptide linker is cleaved by trypsin-like enzymes and the

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disulphide bond (Cys 186-201) is reduced. The cytotoxic domain is subsequently translocated into the cytosol substantially as described above for ricin and elicits ribosomal inhibition and cytotoxicity.

Pseudomonas exotoxin is also a 66kD single-chain toxin protein secreted by Pseudomonas aeruginosa with a similar mechanism of cytotoxicity to that of diphtheria toxin (Pastan, I. et al., Annu. Rev. Biochem. 61:331-354 (1992); Ogata, M. et al., J. Biol. Chem. 267:25396-25401 (1992); Vagil, M.L. et al., Infect. Immunol. 16:353-361 (1977)). Pseudomonas exotoxin consists of 3 conjoint functional domains. The first domain Ia (amino acids 1-252) is responsible for cell binding and toxin endocytosis, a second domain II (amino acids 253-364) is responsible for toxin translocation from the endocytic vesicle to the cytosol, and a third domain III (amino acids 400-613) is responsible for protein synthesis inhibition and cytotoxicity. After Pseudomonas exotoxin enters the cell, the liberation of the cytotoxic domain is effected by both proteolytic cleavage of a polypeptide sequence in the second domain (near Arg 279) and the reduction of the disulphide bond (Cys 265-287) in the endocytic vesicles. In essence, the overall pathway to cytotoxicity is analogous to diphtheria toxin with the exception that the toxin translocation domain in Pseudomonas exotoxin is structurally distinct.

Other toxins possessing distinct functional domains for cytotoxicity and cell binding/toxin translocation include abrin, modeccin and volkensin (Sandvig, K. et al., *Biochem. Soc. Trans.* 21:707-711 (1993)). Some toxins such as Shiga toxin and cholera toxin also have multiple polypeptide chains responsible for receptor binding and endocytosis.

The ricin gene has been cloned and sequenced, and the X-ray crystal structures of the A and B chains have been described (Rutenber, E. et al. *Proteins* 10:240-250 (1991); Weston et al., *Mol. Bio.* 244:410-422, 1994; Lamb and Lord, *Eur. J. Biochem.* 14:265 (1985); Halling, K. et al. *Nucleic Acids Res.* 13:8019 (1985)). Similarly, the genes for

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diptheria toxin and *Pseudomonas* exotoxin have been cloned and sequenced, and the 3-dimensional structures of the toxin proteins have been elucidated and described (Columblatti, M. et al., *J. Biol. Chem.* 261:3030-3035 (1986); Allured, V.S. et al., *Proc. Natl. Acad. Sci. USA* 83:1320-1324 (1986); Gray, G.L. et al., *Proc. Natl. Acad. Sci. USA* 81:2645-2649 (1984); Greenfield, L. et al., *Proc. Natl. Acad. Sci. USA* 80:6853-6857 (1983); Collier, R.J. et al., *J. Biol. Chem.* 257:5283-5285 (1982)).

The potential of bacterial and plant toxins for inhibiting mammalian retroviruses, particularly acquired immunodeficiency syndrome (AIDS), has been investigated. Bacterial toxins such as *Pseudomonas* exotoxin-A and subunit A of diphtheria toxin; dual chain ribosomal inhibitory plant toxins such as ricin, and single chain ribosomal inhibitory proteins such as trichosanthin and pokeweed antiviral protein have been used for the elimination of HIV infected cells (Olson et al., *AIDS Res. and Human Retroviruses* 7:1025-1030 (1991)). The high toxicity of these toxins for mammalian cells, combined with a lack of specificity of action poses a major problem to the development of pharmaceuticals incorporating the toxins, such as immunotoxins.

Due to their extreme toxicity there has been much interest in making ricin-based immunotoxins as therapeutic agents for specifically destroying or inhibiting infected or tumourous cells or tissues (Vitetta et al., Science 238:1098-1104(1987)). An immunotoxin is a conjugate of a specific cell binding component, such as a monoclonal antibody or growth factor and the toxin in which the two protein components are covalently linked. Generally, the components are chemically coupled. However, the linkage may also be a peptide or disulfide bond. The antibody directs the toxin to cell types presenting a specific antigen thereby providing a specificity of action not possible with the natural toxin. Immunotoxins have been made both with the entire ricin molecule (i.e. both chains) and with the ricin A chain alone (Spooner et al., Mol. Immunol. 31:117-125, (1994)).

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Immunotoxins made with the ricin dimer (IT-Rs) are more potent toxins than those made with only the A chain (IT-As). The increased toxicity of IT-Rs is thought to be attributed to the dual role of the B chains in binding to the cell surface and in translocating the A chain to the cytosolic compartment of the target cell (Vitetta et al., Science 238:1098-1104 (1987); Vitetta & Thorpe, Seminars in Cell Biology 2:47-58 (1991)). However, the presence of the B chain in these conjugates also promotes the entry of the immunotoxin into nontarget cells. Even small amounts of B chain may override the specificity of the cell-binding component as the B chain will bind nonspecifically to galactose associated with N-linked carbohydrates, which is present on most cells. IT-As are more specific and safer to use than IT-Rs. However, in the absence of the B chain the A chain has greatly reduced toxicity. Due to the reduced potency of IT-As as compared to IT-Rs, large doses of IT-As must be administered to patients. The large doses frequently cause immune responses and production of neutralizing antibodies in patients (Vitetta et al., Science 238:1098-1104 (1987)). IT-As and IT-Rs both suffer from reduced toxicity as the A chain is not released from the conjugate into the target cell cytoplasm.

A number of immunotoxins have been designed to recognize antigens on the surfaces of tumour cells and cells of the immune system (Pastan et al., Annals New York Academy of Sciences 758:345-353 (1995)). A major problem with the use of such immunotoxins is that the antibody component is its only targeting mechanism and the target antigen is often found on non-target cells (Vitetta et al., Immunology Today 14:252-259 (1993)). Also, the preparation of a suitable specific cell binding component may be problematic. For example, antigens specific for the target cell may not be available and many potential target cells and infective organisms can alter their antigenic make up rapidly to avoid immune recognition. In view of the extreme toxicity of proteins such as ricin, the lack of

specificity of the immunotoxins may severely limit their usefulness as therapeutics for the treatment of cancer and infectious diseases.

The insertion of intramolecular protease cleavage sites between the cytotoxic and cell-binding components of a toxin can mimic the way that the natural toxin is activated. European patent application no. 466,222 describes the use of maize-derived pro-proteins which can be converted into active form by cleavage with extracellular blood enzymes such as factor Xa, thrombin or collagenase. Garred, O. et al. (J. Biol. Chem. 270:10817-10821 (1995)) documented the use of a ubiquitous calcium-dependent serine protease, furin, to activate shiga toxin by cleavage of the trypsin-sensitive linkage between the cytotoxic A-chain and the pentamer of cell-binding B-units. Westby et al. (Bioconjugate Chem. 3:375-381 (1992)) documented fusion proteins which have a specific cell binding component and proricin with a protease sensitive cleavage site specific for factor Xa within the linker sequence. O'Hare et al. (FEBS Lett. 273:200-204 (1990)) also described a recombinant fusion protein of RTA and staphylococcal protein A joined by a trypsin-sensitive cleavage site. In view of the ubiquitous nature of the extracellular proteases utilized in these approaches, such artificial activation of the toxin precursor or immunotoxin does not confer a mechanism for intracellular toxin activation and the problems of target specificity and adverse immunological reactions to the cell-binding component of the immunotoxin remain.

In a variation of the approach of insertion of intramolecular protease cleavage sites on proteins which combine a binding chain and a toxic chain, Leppla, S.H. et al. (Bacterial Protein Toxins zbl.bakt.suppl. 24:431-442 (1994)) suggest the replacement of the native cleavage site of the protective antigen (PA) produced by Bacillus anthracis with a cleavage site that is recognized by cells that contain a particular protease. PA, recognizes, binds, and thereby assists in the internalization of lethal factor (LF) and edema toxin (ET). also produced by Bacillus anthracis. However, this approach is wholly dependent on

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the availability of LF, or ET and PA all being localized to cells wherein the modified PA can be activated by the specific protease. It does not confer a mechanism for intracellular toxin activation and presents a problem of ensuring sufficient quantities of toxin for internalization in target cells.

The *in vitro* activation of a *Staphylococcus*-derived poreforming toxin,  $\alpha$ -hemolysin by extracellular tumour-associated proteases has been documented (Panchel, R.G. et al., *Nature Biotechnology* 14:852-857 (1996)). Artificial activation of  $\alpha$ -hemolysin *in vitro* by said proteases was reported but the actual activity and utility of  $\alpha$ -hemolysin in the destruction of target cells were not demonstrated.

Hemolysin does not inhibit protein synthesis but is a heptameric transmembrane pore which acts as a channel to allow leakage of molecules up to 3 kD thereby disrupting the ionic balances of the living cell. The  $\alpha$ -hemolysin activation domain is likely located on the outside of the target cell (for activation by extracellular proteases). The triggering mechanism in the disclosed hemolysin precursor does not involve the intracellular proteolytic cleavage of 2 functionally distinct domains. Also, the proteases used for the  $\alpha$ -hemolysin activation are ubitquitiously secreted extracellular proteases and toxin activation would not be confined to activation in the vicinity of diseased cells. Such widespread activation of the toxin does not confer target specificity and limits the usefulness of said  $\alpha$ -hemolysin toxin as therapeutics due to systemic toxicity.

A variety of proteases specifically associated with malignancy, viral infections and parasitic infections have been identified and described. For example, cathepsin is a family of serine, cysteine or aspartic endopeptidases and exopeptidases which has been implicated to play a primary role in cancer metastasis (Schwartz, M.K., 30 Clin. Chim. Acta 237:67-78 (1995); Spiess, E. et al., J. Histochem.

Cytochem. 42:917-929 (1994); Scarborough, P.E. et al., Protein Sci. 2:264-276 (1993); Sloane, B.F. et al., Proc. Natl. Acad. Sci. USA 83:2483-2487 (1986); Mikkelsen, T. et al., J. Neurosurge 83:285-290 (1995)). Matrix metalloproteinases (MMPs or matrixins) are zinc-dependent proteinases consisting of collagenases, matrilysin, stromelysins, gelatinases and macrophage elastase (Krane, S.M., Ann. N.Y. Acad. Sci. 732:1-10 (1994); Woessner, J.F., Ann. N.Y. Acad. Sci. 732:11-21 (1994); Carvalho, K. et al., Biochem. Biophys. Res. Comm. 191:172-179 (1993); Nakano, A. et al. J. of Neurosurge, 83:298-307 (1995); Peng, K-W, et al. Human Gene Therapy, 8:729-738 (1997); More, D.H. et al. Gynaecologic Oncology, 65:78-82 These proteases are involved in pathological matrix (1997)). remodeling. Under normal physiological conditions, regulation of matrixin activity is effected at the level of gene expression. Enzymatic activity is also controlled stringently by tissue inhibitors of metalloproteinases (TIMPs) (Murphy, G. et al., Ann. N.Y. Acad. Sci. 732:31-41 (1994)). The expression of MMP genes is reported to be activated in inflammatory disorders (e.g. rheumatoid arthritis) and malignancy.

In malaria, parasitic serine and aspartic proteases are involved in host erythrocyte invasion by the *Plasmodium* parasite and in hemoglobin catabolism by intraerythrocytic malaria (O'Dea, K.P. et al., *Mol. Biochem. Parasitol.* 72:111-119 (1995); Blackman, M.J. et al., *Mol. Biochem. Parasitol.* 62:103-114 (1993); Cooper, J.A. et al., *Mol. Biochem. Parasitol.* 56:151-160 (1992); Goldberg, D.E. et al., *J. Exp. Med.* 173:961-969 (1991)). *Schistosoma mansoni* is also a pathogenic parasite which causes schistosomiasis or bilharzia. Elastinolytic proteinases have been associated specifically with the virulence of this particular parasite (McKerrow, J.H. et al., *J. Biol. Chem.* 260:3703-3707 (1985)).

Welch, A.R. et al. (*Proc. Natl. Acad. Sci. USA* 88:10797-30 10800 (1991)) has described a series of viral proteases which are specifically associated with human cytomegalovirus, human herpesviruses, Epstein-Barr virus, varicella zoster virus-I. and

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infectious laryngotracheitis virus. These proteases possess similar substrate specificity and play an integral role in viral scaffold protein restructuring in capsid assembly and virus maturation. Other viral proteases serving similar functions have also been documented for human T-cell leukemia virus (Blaha, I. et al., FEBS Lett. 309:389-393 (1992); Pettit, S.C. et al., J. Biol. Chem. 266:14539-14547 (1991)), hepatitis viruses (Hirowatari, Y. et al., Anal. Biochem. 225:113-120 (1995); Hirowatari, Y. et al., Arch. Virol. 133:349-356 (1993); Jewell, D.A. et al., Biochemistry 31:7862-7869 (1992)), poliomyelitis virus (Weidner, J.R. et al., Arch. Biochem. Biophys. 286:402-408 (1991)), and human rhinovirus (Long, A.C. et al., FEBS Lett. 258:75-78 (1989)).

Candida yeasts are dimorphic fungi which are responsible for a majority of opportunistic infections in AIDS patients (Holmberg, K. and Myer, R., Scand. J. Infect. Dis. 18:179-192 (1986)). Aspartic proteinases have been associated specifically with numerous virulent strains of Candida including Candida albican, Candida tropicalis, and Candida parapsilosis (Abad-Zapatero, C. et al., Protein Sci. 5:640-652 (1996); Cutfield, S.M. et al., Biochemistry 35:398-410 (1995); Ruchel, R. et al., Zentralbl. Bakteriol. Mikrobiol Hyg. I Abt. Orig. A. 255:537-548 (1983); Remold, H. et al., Biochim. Biophys. Acta 167:399-406 (1968)), and the levels of these enzymes have been correlated with the lethality of the strain (Schreiber, B, et al., Diagn. Microbiol. Infect. Dis. 3:1-5 (1985)).

## **SUMMARY OF THE INVENTION**

25 proteins which are specifically toxic to diseased cells but do not depend for their specificity of action on a specific cell binding component. The recombinant proteins of the invention have an A chain of a ricin-like toxin linked to a B chain by a synthetic linker sequence which may be cleaved specifically by a protease localised in cells or tissues affected by a specific disease to liberate the toxic A chain thereby selectively inhibiting or destroying the diseased cells or tissues. The term diseased

cells as used herein, includes cells affected by cancer, or infected by fungi, or viruses, including retroviruses, or parasites.

Toxin targeting using the recombinant toxic proteins of the invention takes advantage of the fact that many DNA viruses exploit host cellular transport mechanisms to escape immunological destruction. This is achieved by enhancing the retrograde translocation of host major histocompatibility complex (MHC) type I molecules from the endoplasmic reticulum into the cytoplasm (Bonifacino, J.S., Nature 384: 405-406 (1996); Wiertz, E.J. et al., Nature 384: 432-438 (1996)). The facilitation of retrograde transport in diseased cells by the virus can enhance the transcytosis and cytotoxicity of a recombinant toxic protein of the present invention thereby further reducing non-specific cytotoxicity and improving the overall safety of the product.

The recombinant toxic proteins of the present invention may be used to treat diseases including various forms of cancer such as T- and B-cell lymphoproliferative diseases, ovarian cancer, pancreatic cancer, head and neck cancer, squamous cell carcinoma, gastrointestinal cancer, breast cancer, prostate cancer, non small cell lung cancer, malaria, and diverse viral disease states associated with infection with human cytomegalovirus, hepatitis virus, herpes virus, human rhinovirus, infectious laryngotracheitis virus, poliomyelitis virus, or varicella zoster virus.

In one aspect, the present invention provides a purified and isolated nucleic acid having a nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains. The linker sequence is not a native linker sequence of a ricin-like toxin, but rather a synthetic heterologous linker sequence containing a cleavage recognition site for a disease-specific protease. The A and or 30 the B chain may be those of ricin.

In an embodiment, of the invention the cleavage recognition site is the cleavage recognition site for a cancer-associated

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protease. In particular embodiments, the linker amino acid sequence comprises SLLKSRMVPNFN or SLLIARRMPNFN cleaved by cathepsin B; SKLVQASASGVN or SSYLKASDAPDN cleaved by an Epstein-Barr virus protease; RPKPQQFFGLMN cleaved by MMP-3 (stromelysin); SLRPLALWRSFN cleaved by MMP-7 (matrilysin); SPQGIAGQRNFN cleaved by MMP-9; DVDERDVRGFASFL cleaved by a thermolysin-like MMP; SLPLGLWAPNFN cleaved by matrix metalloproteinase 2(MMP-2); SLLIFRSWANFN cleaved by cathespin L; SGVVIATVIVIT cleaved by cathespin D; SLGPQGIWGQFN cleaved by matrix metalloproteinase 1(MMP-1); KKSPGRVVGGSV cleaved by urokinase-type plasminogen 10 activator; PQGLLGAPGILG cleaved by membrane type 1 matrixmetalloproteinase (MT-MMP); HGPEGLRVGFYESDVMGRGHARLVHVEEPHT cleaved by stromelysin 3 (or MMP-11), thermolysin, fibroblast collagenase and stromelysin-1; GPQGLAGQRGIV cleaved by matrix metalloproteinase 13 (collagenase-15 3); GGSGQRGRKALE cleaved by tissue-type plasminogen activator(tPA); SLSALLSSDIFN cleaved by human prostate-specific antigen; SLPRFKIIGGFN cleaved by kallikrein (hK3); SLLGIAVPGNFN cleaved by neutrophil elastase; and FFKNIVTPRTPP cleaved by calpain (calcium activated neutral protease). The nucleic acid sequences for 20 ricin A and B chains with each of the linker sequences are shown in Figures 2D, 35C, 3D, 4D, 5D, 6D, 16D, 17D, 34C, 36C, 37C, 38C, 39C, 40C, 41C, 42C, 43C, 44C, 45C, 46C and 47C, respectively.

In another embodiment, the cleavage recognition site is the cleavage recognition site for a protease associated with the malaria parasite, *Plasmodium falciparum*. In particular embodiments, the linker amino acid sequence comprises QVVQLQNYDEED; LPIFGESEDNDE; QVVTGEAISVTM; ALERTFLSFPTN or KFQDMLNISQHQ. The nucleic nucleotide sequences for ricin A and B chains with each of the linker sequences are shown in Figures 7D, 8D, 9D, 10D, and 11D.

In a another embodiment, the cleavage recognition site is the cleavage recognition site for a viral protease. The linker sequences preferably comprise the sequence Y-X-Y-A-Z wherein X is valine or leucine, Y is a polar amino acid, and Z is serine, asparagine or valine. In particular embodiments, the linker amino acid sequence comprises SGVVNASCRLAN or SSYVKASVSPEN cleaved by a human cytomegalovirus protease; SALVNASSAHVN or STYLQASEKFKN cleaved by a herpes simplex 1 virus protease; SSILNASVPNFN cleaved by a human herpes virus 6 protease; SQDVNAVEASSN or SVYLQASTGYGN cleaved by a varicella zoster virus protease; or SKYLQANEVITN cleaved by an infectious laryngotracheitis virus protease. The nucleic nucleotide sequences for ricin A and B chains with each of the linker sequences are shown in Figures 12D, 13D, 14D, 15D, 18D, 19D, 20D, and 22D.

In another embodiment, the cleavage recognition site is the cleavage recognition site for a hepatitis A viral protease. In particular embodiments, the linker amino acid sequence comprises SELRTQSFSNWN or SELWSQGIDDDN cleaved by a hepatitis A virus protease. The nucleic nucleotide sequences for ricin A and B chains with each of the linker sequences are shown in Figures 23D or 24D.

In another embodiment, the cleavage recognition site is the cleavage recognition site for a hepatitis C viral protease. In particular embodiments, the linker amino acid sequence comprises DLEVVTSTWVFN, DEMEECASHLFN, EDVVCCSMSYFN or KGWRLLAPITAY cleaved by a hepatitis C virus protease. The nucleic nucleotide sequences for ricin A and B chains with each of the linker sequences are shown in Figures 30C, 31C, 32C and 33C.

In another embodiment, the cleavage recognition site is the cleavage recognition site for a *Candida* fungal protease. In particular embodiments, the linker amino acid sequence is SKPAKFFRLNFN, SKPIEFFRLNFN or SKPAEFFALNFN cleaved by *Candida* aspartic

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protease. The nucleic nucleotide sequences for ricin A and B chains with the first linker sequence are shown in Figures 25D.

The present invention also provides a plasmid incorporating the nucleic acid of the invention. In an embodiment, the plasmid has the restriction map as shown in Figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A, 10A, 11A, 12A, 13A, 14A, 15A, 16A, 17A, 18A, 19A, 20A, 21A, 22A, 23A, 24A, or 25A.

In another embodiment, the present invention provides a baculovirus transfer vector incorporating the nucleic acid of the invention. In particular embodiments, the invention provides a baculovirus transfer vector having the DNA sequence as shown in Figure 1.

In a further embodiment, the present invention provides a baculovirus transfer vector incorporating the nucleic acid of the invention. In particular embodiments, the invention provides a baculovirus transfer vector having the restriction map as shown in Figures 2C, 3C, 4C, 5C, 6C, 7C, 8C, 9C, 10C, 11C, 12C, 13C, 14C, 15C, 16C, 17C, 18C, 19C, 20C, 21C, 22C, 23C, 24C, 25C, 30A, 31A, 32A, 33A, 34A, 35A, 36A, 37A, 38A, 39A, 40A, 41A, 42A, 43A, 44A, 45A, 46A, or 47A. or having the DNA sequence as shown in Figure 1.

In a further aspect, the present invention provides a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for a disease-specific protease (e.g., a cancer, viral, parasitic, or fungal protease). The A and/or the B chain may be those of ricin. In an embodiment, the cleavage recognition site is the cleavage recognition site for a cancer, viral or parasitic protease substantially as described above. In a particular embodiment, the cancer is T-cell or B-cell lymphoproliferative disease. In another particular embodiment, the virus is human cytomegalovirus, Epstein-Barr virus, hepatitis virus, herpes virus, human rhinovirus, infectious

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laryngotracheitis virus, poliomyelitis virus, or varicella zoster virus. In a further particular embodiment, the parasite is *Plasmodium* falciparum.

In a further aspect, the invention provides a pharmaceutical composition for treating a fungal infection, such as *Candida*, in a mammal comprising the recombinant protein of the invention and a pharmaceutically acceptable carrier, diluent or excipient.

In yet another aspect, the invention provides a method of inhibiting or destroying cells affected by a disease, which cells are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease, comprising the steps of preparing a recombinant protein of the invention having a heterologous linker sequence which contains a cleavage recognition site for the disease-specific protease and administering the recombinant protein to the cells. In an embodiment, the cancer is T-cell or B-cell lymphoproliferative disease, ovarian cancer, pancreatic cancer, head and neck cancer, squamous cell carcinoma, gastrointestinal cancer, breast cancer, prostate cancer, non small cell lung cancer. In another embodiment, the virus is human cytomegalovirus, Epstein-Barr virus, hepatitis virus, herpes virus, human rhinovirus, human T-cell leukemia virus, infectious laryngotracheitis virus, poliomyelitis virus, or varicella zoster virus. In another embodiment, the parasite is Plasmodium falciparum.

The present invention also relates to a method of treating a mammal with disease wherein cells affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease by administering an effective amount of one or more recombinant proteins of the invention to said mammal.

Still further, a process is provided for preparing a pharmaceutical for treating a mammal with disease wherein cells

affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease comprising the steps of preparing a purified and isolated nucleic acid having a nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for the disease-specific protease; introducing the nucleic acid into a host cell; expressing the nucleic acid in the host cell to obtain a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains wherein the linker sequence contains the cleavage recognition site for the disease-specific protease; and suspending the protein in a pharmaceutically acceptable carrier, diluent or excipient.

In an embodiment, a process is provided for preparing a pharmaceutical for treating a mammal with disease wherein cells affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite each of which has a specific protease comprising the steps of identifying a cleavage recognition site for the protease; preparing a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains wherein the linker sequence contains the cleavage recognition site for the protease and suspending the protein in a pharmaceutically acceptable carrier, diluent or excipient.

In a further aspect, the invention provides a pharmaceutical composition for treating for treating a mammal with disease wherein cells affected by the disease are associated with a disease specific protease, including cancer or infection with a virus, fungus, or a parasite comprising the recombinant protein of the invention and a pharmaceutically acceptable carrier, diluent or excipient.

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Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

### **DESCRIPTION OF THE DRAWINGS**

The invention will be better understood with reference to the drawings in which:

Figure 1 shows the DNA sequence of the baculovirus transfer vector, pVL1393;

Figure 2A summarizes the cloning strategy used to generate the pAP-213 construct;

Figure 2B shows the nucleotide sequence of the Cathepsin B linker regions of pAP-213;

Figure 2C shows the subcloning of the Cathepsin B linker variant into a baculovirus transfer vector;

Figure 2D shows the DNA sequence of the pAP-214 insert containing ricin and the Cathepsin B linker;

Figure 3A summarizes the cloning strategy used to generate the pAP-215 construct;

Figure 3B shows the nucleotide sequence of the MMP-3 linker regions of pAP-215;

Figure 3C shows the subcloning of the MMP-3 linker variant into a baculovirus transfer vector;

Figure 3D shows the DNA sequence of the pAP-216 insert containing ricin and the MMP-3 linker;

Figure 4A summarizes the cloning strategy used to 30 generate the pAP-217 construct;

Figure 4B shows the nucleotide sequence of the MMP-7 linker regions of pAP-217;

Figure 4C shows the subcloning of the MMP-7 linker variant into a baculovirus transfer vector;

Figure 4D shows the DNA sequence of the pAP-218 insert containing ricin and the MMP-7 linker;

Figure 5A summarizes the cloning strategy used to generate the pAP-219 construct;

Figure 5B shows the nucleotide sequence of the MMP-9 linker regions of pAP-219;

Figure 5C shows the subcloning of the MMP-9 linker variant into a baculovirus transfer vector;

Figure 5D shows the DNA sequence of the pAP-220 insert containing ricin and the MMP-9 linker.

Figure 6A summarizes the cloning strategy used to generate the pAP-221 construct;

Figure 6B shows the nucleotide sequence of the thermolysin-like MMP linker regions of pAP-221;

Figure 6C shows the subcloning of the thermolysin-like MMP linker variant into a baculovirus transfer vector.

Figure 6D shows the DNA sequence of the pAP-222 insert containing ricin and the thermolysin-like MMP linker;

Figure 7A summarizes the cloning strategy used to generate the pAP-223 construct;

Figure 7B shows the nucleotide sequence of the Plasmodium falciparum-A linker regions of pAP-223;

Figure 7C shows the subcloning of the Plasmodium falciparum-A linker variant into a baculovirus transfer vector;

Figure 7D shows the DNA sequence of the pAP-224 insert containing ricin and the Plasmodium falciparum-A linker;

Figure 8A summarizes the cloning strategy used to 30 generate the pAP-225 construct;

Figure 8B shows the nucleotide sequence of the Plasmodium falciparum-B linker regions of pAP-225;

Figure 8C shows the subcloning of the Plasmodium falciparum-B linker variant into a baculovirus transfer vector;

Figure 8D shows the DNA sequence of the pAP-226 insert containing ricin and the Plasmodium falciparum-B linker;

Figure 9A summarizes the cloning strategy used to generate the pAP-227 construct;

Figure 9B shows the nucleotide sequence of the Plasmodium falciparum-C linker regions of pAP-227;

Figure 9C shows the subcloning of the Plasmodium 10 falciparum-C linker variant into a baculovirus transfer vector;

Figure 9D shows the DNA sequence of the pAP-228 insert containing ricin and the Plasmodium falciparum-C linker;

Figure 10A summarizes the cloning strategy used to generate the pAP-229 construct;

Figure 10B shows the nucleotide sequence of the Plasmodium falciparum-D linker regions of pAP-229;

Figure 10C shows the subcloning of the Plasmodium falciparum-D linker variant into a baculovirus transfer vector;

Figure 10D shows the DNA sequence of the pAP-230 insert containing ricin and the Plasmodium falciparum-D linker;

Figure 11A summarizes the cloning strategy used to generate the pAP-231 construct;

Figure 11B shows the nucleotide sequence of the Plasmodium falciparum-E linker regions of pAP-231;

Figure 11C shows the subcloning of the Plasmodium falciparum-E linker variant into a baculovirus transfer vector;

Figure 11D shows the DNA sequence of the pAP-232 insert containing ricin and the Plasmodium falciparum-E linker;

Figure 12A summarizes the cloning strategy used to 30 generate the pAP-233 construct;

Figure 12B shows the nucleotide sequence of the HSV-A linker regions of pAP-233;

Figure 12C shows the subcloning of the HSV-A linker variant into a baculovirus transfer vector;

Figure 12D shows the DNA sequence of the pAP-234 insert containing ricin and the HSV-A linker;

Figure 13A summarizes the cloning strategy used to generate the pAP-235 construct;

Figure 13B shows the nucleotide sequence of the HSV-B linker regions of pAP-235;

Figure 13C shows the subcloning of the HSV-B linker 10 variant into a baculovirus transfer vector;

Figure 13D shows the DNA sequence of the pAP-236 insert containing ricin and the HSV-B linker;

Figure 14A summarizes the cloning strategy used to generate the pAP-237 construct;

Figure 14B shows the nucleotide sequence of the VZV-A linker regions of pAP-237;

Figure 14C shows the subcloning of the VZV-A linker variant into a baculovirus transfer vector;

Figure 14D shows the DNA sequence of the pAP-238 insert containing ricin and the VZV-A linker;

Figure 15A summarizes the cloning strategy used to generate the pAP-239 construct;

Figure 15B shows the nucleotide sequence of the VZV-B linker regions of pAP-239;

Figure 15C shows the subcloning of the VZV-B linker variant into a baculovirus transfer vector;

Figure 15D shows the DNA sequence of the pAP-240 insert containing ricin and the VZV-B linker;

Figure 16A summarizes the cloning strategy used to 30 generate the pAP-241 construct;

Figure 16B shows the nucleotide sequence of the EBV-A linker regions of pAP-241;

Figure 16C shows the subcloning of the EBV-A linker variant into a baculovirus transfer vector;

Figure 16D shows the DNA sequence of the pAP-242 insert containing ricin and the EBV-A linker;

Figure 17A summarizes the cloning strategy used to generate the pAP-243 construct;

Figure 17B shows the nucleotide sequence of the EBV-B linker regions of pAP-243;

Figure 17C shows the subcloning of the EBV-B linker 10 variant into a baculovirus transfer vector;

Figure 17D shows the DNA sequence of the pAP-244 insert containing ricin and the EBV-B linker;

Figure 18A summarizes the cloning strategy used to generate the pAP-245 construct;

Figure 18B shows the nucleotide sequence of the CMV-A linker regions of pAP-245;

Figure 18C shows the subcloning of the CMV-A linker variant into a baculovirus transfer vector;

Figure 18D shows the DNA sequence of the pAP-246 20 insert containing ricin and the CMV-A linker;

Figure 19A summarizes the cloning strategy used to generate the pAP-247 construct;

Figure 19B shows the nucleotide sequence of the CMV-B linker regions of pAP-247;

Figure 19C shows the subcloning of the CMV-B linker variant into a baculovirus transfer vector;

Figure 19D shows the DNA sequence of the pAP-248 insert containing ricin and the CMV-B linker.

Figure 20A summarizes the cloning strategy used to 30 generate the pAP-249 construct;

Figure 20B shows the nucleotide sequence of the HHV-6 linker regions of pAP-249;

Figure 20C shows the subcloning of the HHV-6 linker variant into a baculovirus transfer vector;

Figure 20D shows the DNA sequence of the pAP-250 insert containing ricin and the HHV-6 linker;

Figure 21 shows the amino acid sequences of the wild type ricin linker and cancer protease-sensitive amino acid linkers contained in pAP-213 to pAP-222 and linkers pAP-241 to pAP-244;

Figure 22A summarizes the cloning strategy used to generate the pAP-253 construct;

Figure 22B shows the nucleotide sequence of the ILV linker regions of pAP-253;

Figure 22C shows the subcloning of the ILV linker variant into a baculovirus transfer vector;

Figure 22D shows the DNA sequence of the pAP-254 insert containing ricin and the ILV linker;

Figure 23A summarizes the cloning strategy used to generate the pAP-257 construct;

Figure 23B shows the nucleotide sequence of the HAV-A linker regions of pAP-257;

Figure 23C shows the subcloning of the HAV-A linker variant into a baculovirus transfer vector;

Figure 23D shows the DNA sequence of the pAP-258 insert containing ricin and the HAV-A linker;

Figure 24A summarizes the cloning strategy used to 25 generate the pAP-255 construct;

Figure 24B shows the nucleotide sequence of the HAV-B linker regions of pAP-255;

Figure 24C shows the subcloning of the HAV-B linker variant into a baculovirus transfer vector;

Figure 24D shows the DNA sequence of the pAP-256 insert containing ricin and the HAV-B linker;

Figure 25A summarizes the cloning strategy used to generate the pAP-259 construct;

Figure 25B shows the nucleotide sequence of the CAN linker regions of pAP-259;

Figure 25C shows the subcloning of the CAN linker variant into a baculovirus transfer vector;

Figure 25D shows the DNA sequence of the pAP-260 insert containing ricin and the CAN linker;

Figure 26 shows the amino acid sequences of the wild type ricin linker and *Plasmodium falciparum* protease-sensitive amino acid linkers contained in linkers pAP-223 to pAP-232;

Figure 27 shows the amino acid sequences of the wild type ricin linker and the viral protease-sensitive amino acid linkers contained in pAP-233 to pAP-240, pAP-245-pAP-248, pAP-253 to pAP-258;

Figure 28 shows the amino acid sequences of the wild type ricin linker and the *Candida* aspartic protease-sensitive amino acid linker contained in pAP-259 to pAP-264;

Figure 29 describes an alternative mutagenesis and subcloning strategy to provide a baculovirus transfer vector containing the ricin-like toxin variant gene; and

Figure 30A summarizes the cloning strategy used to generate the pAP-262 construct;

Figure 30B shows the nucleotide sequence of the HCV-A linker region of pAP-262;

Figure 30C shows the DNA sequence of the pAP-262 insert;

Figure 30D shows the amino acid sequence comparison of mutant preproricin linker region HCV-A to wild type;

Figure 31A summarizes the cloning strategy used to generate the pAP-264 construct;

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Figure 31B shows the nucleotide sequence of the HCV-B linker region of pAP-264;

Figure 31C shows the DNA sequence of the pAP-264 insert;

Figure 31D shows the amino acid sequence comparison of mutant preproricin linker region HCV-B to wild type;

Figure 32A summarizes the cloning strategy used to generate the pAP-266 construct;

Figure 32B shows the nucleotide sequence of the HCV-C linker region of pAP-266;

Figure 32C shows the DNA sequence of the pAP-266 insert;

Figure 32D shows the amino acid sequence comparison of mutant preproricin linker region HCV-C to wild type;

Figure 33A summarizes the cloning strategy used to generate the pAP-268 construct;

Figure 33B shows the nucleotide sequence of the HCV-D linker region of pAP-268;

Figure 33C shows the DNA sequence of the pAP-268 20 insert;

Figure 33D shows the amino acid sequence comparison of mutant preproricin linker region HCV-D to wild type;

Figure 34A summarizes the cloning strategy used to generate the pAP-270 construct;

Figure 34B shows the nucleotide sequence of the MMP-2 linker region of pAP-270;

Figure 34C shows the DNA sequence of the pAP-270 insert;

Figure 34D shows the amino acid sequence comparison of mutant preproricin linker region of MMP-2 to wild type;

Figure 35A summarizes the cloning strategy used to generate the pAP-272 construct;

Figure 35B shows the nucleotide sequence of the Cathepsin B (Site 2) linker region of pAP-272;

Figure 35C shows the DNA sequence of the pAP-272 insert;

Figure 35D shows the amino acid sequence comparison of mutant preproricin linker region of Cathepsin B (Site 2) to wild type;

Figure 36A summarizes the cloning strategy used to generate the pAP-274 construct;

Figure 36B shows the nucleotide sequence of the 10 Cathepsin L linker region of pAP-274;

Figure 36C shows the DNA sequence of the pAP-274 insert;

Figure 36D shows the amino acid sequence comparison of mutant preproricin linker region of Cathepsin L to wild type;

Figure 37A summarizes the cloning strategy used to generate the pAP-276 construct;

Figure 37B shows the nucleotide sequence of the Cathepsin D linker region of pAP-276;

Figure 37C shows the DNA sequence of the pAP-276 20 insert;

Figure 37D shows the amino acid sequence comparison of mutant preproricin linker region of Cathepsin D to wild type;

Figure 38A summarizes the cloning strategy used to generate the pAP-278 construct;

Figure 38B shows the nucleotide sequence of the MMP-1 linker region of pAP-278;

Figure 38C shows the DNA sequence of the pAP-278 insert;

Figure 38D shows the amino acid sequence comparison of mutant preproricin linker region of MMP-1 to wild type;

Figure 39A summarizes the cloning strategy used to generate the pAP-280 construct;

Figure 39B shows the nucleotide sequence of the Urokinase-Type Plasminogen Activator linker region of pAP-280;

Figure 39C shows the DNA sequence of the pAP-280 insert;

Figure 39D shows the amino acid sequence comparison of mutant preproricin linker region of Urokinase-Type Plasminogen Activator to wild type;

Figure 40A summarizes the cloning strategy used to generate the pAP-282 construct;

Figure 40B shows the nucleotide sequence of the MT-MMP linker region of pAP-282;

Figure 40C shows the DNA sequence of the pAP-282 insert;

Figure 40D shows the amino acid sequence comparison of mutant preproricin linker region of MT-MMP to wild type;

Figure 41A summarizes the cloning strategy used to generate the pAP-284 construct;

Figure 41B shows the nucleotide sequence of the MMP-11 linker region of pAP-284;

Figure 41C shows the DNA sequence of the pAP-284 insert;

Figure 41D shows the amino acid sequence comparison of mutant preproricin linker region of MMP-11 to wild type;

Figure 42A summarizes the cloning strategy used to 25 generate the pAP-286 construct;

Figure 42B shows the nucleotide sequence of the MMP-13 linker region of pAP-286;

Figure 42C shows the DNA sequence of the pAP-286 insert;

Figure 42D shows the amino acid sequence comparison of mutant preproricin linker region of MMP-13 to wild type;

Figure 43A summarizes the cloning strategy used to generate the pAP-288 construct;

Figure 43B shows the nucleotide sequence of the Tissuetype Plasminogen Activator linker region of pAP-288;

Figure 43C shows the DNA sequence of the pAP-288 insert;

Figure 43D shows the amino acid sequence comparison of mutant preproricin linker region of Tissue-type Plasminogen Activator to wild type;

Figure 44A summarizes the cloning strategy used to generate the pAP-290 construct;

Figure 44B shows the nucleotide sequence of the human Prostate-Specific Antigen linker region of pAP-290;

Figure 44C shows the DNA sequence of the pAP-290 insert;

Figure 44D shows the amino acid sequence comparison of mutant preproricin linker region of the human Prostate-Specific Antigen to wild type;

Figure 45A summarizes the cloning strategy used to 20 generate the pAP-292 construct;

Figure 45B shows the nucleotide sequence of the kallikrein linker region of pAP-292;

Figure 45C shows the DNA sequence of the pAP-292 insert;

Figure 45D shows the amino acid sequence comparison of mutant preproricin linker region of the kallikrein to wild type;

Figure 46A summarizes the cloning strategy used to generate the pAP-294 construct;

Figure 46B shows the nucleotide sequence of the 30 neutrophil elastase linker region of pAP-294;

Figure 46C shows the DNA sequence of the pAP-294 insert;

Figure 46D shows the amino acid sequence comparison of mutant preproricin linker region of neutrophil elastase to wild type;

Figure 47A summarizes the cloning strategy used to generate the pAP-296 construct;

Figure 47B shows the nucleotide sequence of the calpain linker region of pAP-296;

Figure 47C shows the DNA sequence of the pAP-296 insert;

Figure 47D shows the amino acid sequence comparison of 10 mutant preproricin linker region of calpain to wild type;

Figure 48 is a blot showing cleavage of pAP-214 by

Figure 49 is a blot showing cleavage of pAP-220 with MMP-9;

Figure 50 is a blot showing activation of pAP-214; and Figure 51 is a blot showing activation of pAP-220.

Figure 52 is a blot showing cleavage of pAP-248 with

HCMV.

Cathepsin B;

Figure 53 is a blot showing activation of pAP-248.

Figure 54 is a blot showing cleavage of pAP-256 by HAV 3C.

Figure 55 is a blot showing activation of pAP-256.

Figure 56 is a semi-logithmic graph illustrating the cytotoxicity to COS-1 cells of undigested pAP-214 and pAP-214 digested with Cathepsin B.

Figure 57 is a semi-logithmic graph illustrating the cytotoxicity of pAP-220 digested with MMP-9 compared to freshly thawed pAP-220 and ricin on COS-1 cells.

Figure 58 is a blot showing cleavage of pAP-270 with

30 MMP-2.

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Figure 59 is a blot showing activation of pAP-270. Figure 60 is a blot showing cleavage of pAP-288 by t-PA.

Figure 61 is a blot showing activation of pAP-288.

Figure 62 is a blot showing cleavage of pAP-294 with human neutrophil elastase.

Figure 63 is a blot showing activation of pAP-294.

Figure 64 is a blot showing cleavage of pAP-296 with calpain.

Figure 65 is a blot showing activation of pAP-296.

Figure 66 is a blot showing cleavage of pAP-222 with

MMP-2.

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Figure 67 is a blot showing activation of pAP-222.

### **DETAILED DESCRIPTION OF THE INVENTION**

### Nucleic Acid Molecules of the Invention

As mentioned above, the present invention relates to novel nucleic acid molecules comprising a nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains. The heterologous linker sequence contains a cleavage recognition site for a disease-specific protease (e.g. a viral protease, parasitic protease, cancer-associated protease, or a fungal protease).

The term "isolated and purified" as used herein refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. An "isolated and purified" nucleic acid is also substantially free of sequences which naturally flank the nucleic acid (*i.e.* sequences located at the 5' and 3' ends of the nucleic acid) from which the nucleic acid is derived. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded.

The term "linker sequence" as used herein refers to an internal amino acid sequence within the protein encoded by the nucleic acid molecule of the invention which contains residues linking the A and B chain so as to render the A chain incapable of exerting its toxic

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effect, for example catalytically inhibiting translation of a eukaryotic ribosome. By heterologous is meant that the linker sequence is not a sequence native to the A or B chain of a ricin-like toxin or precursor thereof. However, preferably, the linker sequence may be of a similar length to the linker sequence of a ricin-like toxin and should not interfere with the role of the B chain in cell binding and transport into the cytoplasm. When the linker sequence is cleaved the A chain becomes active or toxic.

The nucleic acid molecule of the invention is cloned by subjecting a preproricin cDNA clone to site-directed mutagenesis in order to generate a series of variants differing only in the sequence between the A and B chains (linker region). Oligonucleotides, corresponding to the extreme 5' and 3' ends of the preproricin gene are synthesized and used to PCR amplify the gene. Using the cDNA sequence for preproricin (Lamb et al., Eur. J. Biochem. 145:266-270 (1985)), several oligonucleotide primers are designed to flank the start and stop codons of the preproricin open reading frame.

The preproricin cDNA is amplified using the upstream primer Ricin-99 or Ricin-109 and the downstream primer Ricin1729C with Vent DNA polymerase (New England Biolabs) using standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)). The purified PCR fragment encoding the preproricin cDNA is then ligated into an Eco RI-digested pBluescript II SK plasmid (Stratagene), and is used to transform competent XL1-Blue cells (Stratagene). The cloned PCR product containing the putative preproricin gene is confirmed by DNA sequencing of the entire cDNA clone. The sequences and location of oligonucleotide primers used for sequencing are shown in Table 1.

The preproricin cDNA clone is subjected to site directed mutagenesis in order to generate a series of variants differing only in the sequence between the A and B chains (linker region). The wild-type

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preproricin linker region is replaced with the heterogenous linker sequences that are cleaved by the various disease-specific proteases as shown in Figures 21, 26, 27, 28, and Part D of Figures 30-47. Linker identification as used herein in connection with the sequences provided in these figures have been assigned the sequence ID numbers as discussed below.

The linker regions of the variants encode a cleavage recognition sequence for a disease-specific protease associated with for example, cancer, viruses, parasites, or fungii. The mutagenesis and cloning strategy used to generate the disease-specific protease-sensitive linker variants are summarized in Part A of Figures 2-20, and Part A of Figures 22-25. The first step involves a DNA amplification using a set of mutagenic primers in combination with the two flanking primers Richin-99Eco or Ricin-109Eco and Ricin1729C Pst I. Restriction digested PCR fragments are gel purified and then ligated with PBluescript SK which has been digested with Eco RI and Pst I. Ligation reactions are used to transform competent XL1-Blue cells (Stratagene). Recombinant clones are identified by restriction digests of plasmid miniprep DNA and the mutant linker sequences are confirmed by DNA sequencing. With respect to the nucleotide sequences and amino acid sequences prepared as a result of the implementation of this strategy the following sequences have been assigned the sequence ID numbers as indicated.

SEQ ID NO. 1 is used herein in connection with the DNA sequence of the baculovirus transfer vector, pVL1393.

The nucleotide sequence of Cathepsin B linker regions of pAP-213 are referred to herein as SEQ ID NO. 2.

The nucleotide sequence of Cathepsin B linker regions of pAP-214 are referred to herein as SEQ ID NO. 3.

The nucleotide sequence of MMP-3 linker regions of pAP-30 215 are referred to herein as SEQ ID NO. 4.

The DNA sequence of the pAP-216 insert containing ricin and the MMP-3 linker are referred to herein as SEQ ID NO. 5.

The nucleotide sequence of MMP-7 linker regions of pAP-217 are referred to herein as SEQ ID NO. 6.

The DNA sequence of the pAP-218 insert containing ricin and the MMP-7 linker are referred to herein as SEQ ID NO. 7.

The nucleotide sequence of MMP-9 linker regions of pAP-219 are referred to herein as SEQ ID NO. 8.

The DNA sequence of the pAP-220 insert containing ricin and the MMP-9 are referred to herein as SEQ ID NO. 9.

The nucleotide sequence of thermolysin-like MMP linker regions of pAP-221 are referred to herein as SEQ ID NO. 10.

The DNA sequence of pAP-222 insert containing ricin and the thermolysin-like MMP linker are referred to herein as SEQ ID NO. 11.

The nucleotide sequence of Plasmodium falciparum-A linker regions of pAP-223 are referred to herein as SEQ ID NO. 12.

The DNA sequence of the pAP-224 insert containing ricin and the Plasmodium falciparum-A linker are referred to herein as SEQ ID NO. 13.

The nucleotide sequence of Plasmodium falciparum-B linker regions of pAP-225 are referred to herein as SEQ ID NO. 14.

The DNA sequence of the pAP-226 insert containing ricin and the Plasmodium falciparum-B linker are referred to herein as SEQ ID NO. 15.

The nucleotide sequence of Plasmodium falciparum-C linker regions of pAP-227 are referred to herein as SEQ ID NO. 16.

The DNA sequence of the pAP-228 insert containing ricin and the Plasmodium falciparum-C linker are referred to herein as SEQ ID NO. 17.

The nucleotide sequence of the the Plasmodium 30 falciparum-D linker regions of pAP-229 is referred to herein as SEQ ID NO. 18.

The DNA sequence of the pAP-230 insert containing ricin and the Plasmodium falciparum-D linker is referred to herein as SEQ ID NO. 19.

The nucleotide sequence of the Plasmodium falciparum-5 E linker regions of pAP-231 is referred to herein as SEQ ID NO. 20.

The DNA sequence of the pAP-232 insert containing ricin and the Plasmodium falciparum-E linker is referred to herein as SEQ ID NO. 21.

The nucleotide sequence of the HSV-A linker regions of pAP-233 is referred to herein as SEQ ID NO. 22.

The DNA sequence of the pAP-234 insert containing ricin and the HSV-A linker is referred to herein as SEQ ID NO. 23.

The nucleotide sequence of the HSV-B linker regions of pAP-235 is referred to herein as SEQ ID NO. 24.

The DNA sequence of the pAP-236 insert containing ricin and the HSV-B linker is referred to herein as SEQ ID NO. 25.

The nucleotide sequence of the VZV-A linker regions of pAP-237 are referred to herein as SEQ ID NO. 26.

The DNA sequence of the pAP-238 insert containing ricin and the VZV-A linker are referred to herein as SEQ ID NO. 27.

The nucleotide sequence of the VZV-B linker regions of PAP-239 is referred to herein as SEQ ID NO. 28.

The DNA sequence of the pAP-240 insert containing ricin and the VZV-B linker is referred to herein as SEQ ID NO. 29.

The nucleotide sequence of the EBV-A linker regions of pAP-241 is referred to herein as SEQ ID NO. 30.

The DNA sequence of the pAP-242 insert containing ricin and the EBV-A linker is referred to herein as SEQ ID NO. 31.

The nucleotide sequence of the EBV-B linker regions of pAP-243 is referred to herein as SEQ ID NO. 32.

The DNA sequence of the pAP-244 insert containing ricin and the EBV-B linker is referred to herein as SEQ ID NO. 33.

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The nucleotide sequence of the CMV-A linker regions of pAP-245 is referred to herein as SEQ ID NO. 34.

The DNA sequence of the pAP-246 insert containing ricin and the CMV-A linker is referred to herein as SEQ ID NO. 35.

The nucleotide sequence of the CMV-B linker regions of pAP-247 is referred to herein as SEQ ID NO. 36.

The DNA sequence of the pAP-248 insert containing ricin and the CMV-B linker is referred to herein as SEQ ID NO. 37.

The nucleotide sequence of the HHV-6 linker regions of pAP-249 is referred to herein as SEQ ID NO. 38.

The DNA sequence of the pAP-250 insert containing ricin and the HHV-6 linker is referred to herein as SEQ ID NO. 39.

The amino acid sequences of the cancer protease-sensitive amino acid linkers contained in the following pAP proteins have the sequence ID numbers as indicated: pAP-213 and pAP-214 (SEQ ID NO. 40); pAP-215 and pAP-216 (SEQ ID NO. 41); pAP-217 and pAP-218; (SEQ ID NO. 42); pAP-219 and pAP-220 (SEQ ID NO. 43); and pAP-221 and pAP-222 (SEQ ID NO. 44).

The amino acid sequences of the following cancer protease-sensitive linkers are referred to herein with the corresponding sequence ID numbers: pAP-241 and pAP-242 (SEQ ID NO. 45); and pAP-243 and pAP-244 (SEQ ID NO. 46).

The nucleotide sequence of the ILV linker regions of pAP-253 is referred to herein as SEQ ID NO. 47.

The DNA sequence of the pAP-254 insert containing ricin and the ILV linker is referred to herein as SEQ ID NO. 48.

The nucleotide sequence of the HAV-A linker regions of pAP-257 is referred to herein as SEQ ID NO. 49.

The DNA sequence of the pAP-258 insert containing ricin and HAV-A linker is referred to herein as SEQ ID NO. 50.

The nucleotide sequence of the HAV-B linker regions of pAP-255 is referred to herein as SEQ ID NO. 51.

The DNA sequence of the pAP-256 insert containing ricin and the HAV-B linker is referred to herein as SEQ ID NO. 52.

The nucleotide sequence of the CAN linker regions of pAP-259 is referred to herein as SEQ ID NO. 53.

The DNA sequence of the pAP-260 insert containing ricin and the CAN linker is referred to herein as SEQ ID NO. 54.

The amino acid sequences of Plasmodium falciparum protease-sensitive linkers are referred to herein by the sequence ID numbers as follows: pAP-223 and pAP-224 (SEQ ID NO 55); pAP-225 and pAP-226 (SEQ ID NO 56); pAP-227 and pAP-228 (SEQ ID NO 57); pAP-229 and pAP-230 (SEQ ID NO 58); and pAP-231 and pAP-232 (SEQ ID NO 59) (see Figure 26).

The amino acid sequences of the viral protease-sensitive linkers which follow are referred to herein by the sequence ID numbers indicated: pAP-233 and pAP 234 (SEQ ID NO 60); pAP-235 and pAP-236 (SEQ ID NO 61); and pAP-249 and pAP-250 (SEQ ID NO 62) (see Figure 27).

The amino acid sequences of the viral protease-sensitive linkers which follow are referred to herein by the sequence ID numbers indicated: pAP-245 and pAP-246 (SEQ ID NO 63); and pAP-247 and pAP-248 (SEQ ID NO 64) (see Figure 27).

The amino acid sequences of the viral protease-sensitive linkers which follow are referred to herein by the sequence ID numbers indicated: pAP-237 and pAP-238 (SEQ ID NO 65); and pAP-239 and pAP-240 (SEQ ID NO 66); pAP-253 and pAP-254 (SEQ ID NO 67); pAP-255 and pAP-256 (SEQ ID NO 68); and pAP-257 and pAP-258 (SEQ ID NO 69) (see Figure 27).

The amino acid sequences of the *Candida* aspartic protease-sensitive linkers are referred to herein by the sequence ID numbers indicated: pAP-259 and pAP-260 (SEQ ID NO 70); pAP-261 and pAP-262 (SEQ ID NO 71); and pAP-263 and pAP-264 (SEQ ID NO 72).

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An alternative mutagenesis and cloning strategy that can be used to generate the disease-specific protease-sensitive linker variants is summarized in Figure 29. The first step of this method involves a DNA amplification using a set of mutagenic primers in combination with the two flanking primers Ricin-109Eco and Ricin1729Pst. Restriction digested PCR fragments (Eco RI and Pst I) are gel purified. Preproricin variants produced from this method can be subcloned directly into the baculovirus transfer vector digested with Eco RI and Pst I and intermediate ligation steps involving pBluescript SK and pSB2 are circumvented. The cloning strategies used to generate disease-specific protease-sensitive linker variants are summarized in Part A of Figures 30 to 47. With respect to the nucleotide sequences and amino acid sequences prepared as a result of the implementation of this strategy the following sequences have been assigned the sequence ID numbers as indicated.

The nucleotide sequence of the HCV-A linker region of pAP-262 is referred to herein as SEQ ID NO. 73.

The DNA sequence of the pAP-262 insert is referred to herein as SEQ ID NO. 74.

The amino acid sequence of the mutant preproricin linker region for HCV-A, pAP-262, is referred to herein as SEQ ID NO. 75.

The nucleotide sequence of the HCV-B linker region of pAP-264 is referred to herein as SEQ ID NO. 76.

The DNA sequence of the pAP-264 insert is referred to herein as SEQ ID NO. 77.

The amino acid sequence of the mutant preproricin linker region for HCV-B, pAP-264, is referred to herein as SEQ ID NO. 78.

The nucleotide sequence of the HCV-C linker region of pAP-266 is referred to herein as SEQ ID NO. 79.

The DNA sequence of the pAP-266 insert is referred to herein as SEQ ID NO. 80.

The amino acid sequence of the mutant preproricin linker region for HCV-C, pAP-266, is referred to herein as SEQ ID NO. 81.

The nucleotide sequence of the HCV-D linker region of pAP-268 is referred to herein as SEQ ID NO. 82.

The DNA sequence of the pAP-268 insert is referred to herein as SEQ ID NO. 83.

The amino acid sequence of the mutant preproricin linker region for HCV-D , pAP-268, is referred to herein as SEQ ID NO. 84.

The nucleotide sequence of the MMP-2 linker region of pAP-270 is referred to herein as SEQ ID NO. 85.

The DNA sequence of the pAP-270 insert is referred to herein as SEQ ID NO. 86.

The amino acid sequence of the mutant preproricin linker region for MMP-2, pAP-270, is referred to herein as SEQ ID NO. 87.

The nucleotide acid sequence of the Cathepsin B (Site 2) linker region of pAP-272 is referred to herein as SEQ ID NO. 88.

The DNA sequence of the pAP-272 insert is referred to herein as SEQ ID NO. 89.

The amino acid sequence of the mutant preproricin linker region for Cathepsin B (Site 2), pAP-272, is referred to herein as SEQ ID NO. 90.

The nucleotide sequence of the Cathepsin L linker region of pAP-274 is referred to herein as SEQ ID NO. 91.

The DNA sequence of the pAP-274 insert is referred to 30 herein as SEQ ID NO. 92.

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The amino acid sequence of the mutant preproricin linker region of Cathepsin L, pAP-274, is referred to herein as SEQ ID NO. 93.

The nucleotide sequence of Cathepsin D linker region of pAP-276 is referred to herein as SEQ ID NO. 94.

The DNA sequence of the pAP-276 insert is referred to herein as SEQ ID NO. 95.

The amino acid sequence of the mutant preproricin linker region for Cathepsin D, pAP-276, is referred to herein as SEQ ID NO. 96.

The nucleotide sequence of the MMP-1 linker region of pAP-278 is referred to herein as SEQ ID NO. 97.

The DNA sequence of the pAP-278 insert is referred to herein as SEQ ID NO. 98.

The amino acid sequence of the mutant preproricin linker region for MMP-1, pAP-278, is referred to herein as SEQ ID NO. 99.

The nucleotide sequence of the Urokinase-Type Plasminogen Activator linker region of pAP-280 is referred to herein as SEQ ID NO. 100.

The DNA sequene of the pAP-280 insert is referred to herein as SEQ ID NO. 101.

The amino acid sequence of the mutant preproricin linker region for Urokinase-Type Plasminogen Activator, pAP-280, is referred to herein as SEQ ID NO. 102.

The nucleotide sequence of MT-MMP linker region of pAP-282 is referred to herein as SEQ ID NO. 103.

The DNA sequence of the pAP-282 insert is referred to herein as SEQ ID NO. 104.

The amino acid sequence of the mutant preproricin linker region for MT-MMP, pAP-282, is referred to herein as SEQ ID NO. 105.

The nucleotide sequence of the MMP-11 linker region of pAP-284 is referred to herein as SEQ ID NO. 106.

The DNA sequence of the pAP-284 insert is referred to herein as SEQ ID NO. 107.

The amino acid sequence of the mutant preproricin linker region for MMP-11, pAP-284, is referred to herein as SEQ ID NO. 108.

The nucleotide sequence of the MMP-13 linker region of pAP-286 is referred to herein as SEQ ID NO. 109.

The DNA sequence of the pAP-286 insert is referred to herein as SEQ ID NO. 110.

The amino acid sequence of the mutant preproricin linker region for MMP-13, pAP-286, is referred to herein as SEQ ID NO. 111.

The nucleotide sequence of the Tissue-type Plasminogen Activator linker region of pAP-288 is referred to herein as SEQ ID NO. 112.

The DNA sequence of the pAP-288 insert is referred to herein as SEQ ID NO. 113.

The amino acid sequence of the mutant preproricin linker region for Tissue-type Plasminogen Activator, pAP-288, is referred to herein as SEQ ID NO. 114.

The nucleotide sequence of the human Prostate-Specific Antigen linker region of pAP-290 is referred to herein as SEQ ID NO. 115.

The DNA sequence of the pAP-290 insert is referred to herein as SEQ ID NO. 116.

The amino acid sequence of the mutant preproricin linker region for the human Prostate-Specific Antigen, pAP-290, is referred to herein as SEQ ID NO. 117.

The nucleotide sequence of the kallikrein linker region of pAP-292 is referred to herein as SEQ ID NO. 118.

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The DNA sequence of the pAP-292 insert is referred to herein as SEQ ID NO. 119.

The amino acid sequence of the mutant preproricin linker region for the kallikrein, pAP-292, is referred to herein as SEQ ID NO. 120.

The nucleotide sequence of the neutrophil elastase linker region of pAP-294 is referred to herein as SEQ ID NO. 121.

The DNA sequence of the pAP-294 insert is referred to herein as SEQ ID NO. 122.

The amino acid sequence of the mutant preproricin linker region for neutrophil elastase, pAP-294, is referred to herein as SEQ ID NO. 123.

The nucleotide sequence of the calpain linker region of pAP-296 is referred to herein as SEQ ID NO. 124.

The DNA sequence of the pAP-296 insert is referred to herein as SEQ ID NO. 125.

The amino acid sequence of the mutant preproricin linker region for calpain, pAP-296, is referred to herein as SEQ ID NO. 126.

The amino acid sequence of the wild type linker region is referred to herein as SEQ ID NO. 127.

The nucleic acid molecule of the invention has sequences encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease. The nucleic acid may be expressed to provide a recombinant protein having an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease.

The nucleic acid molecule may comprise the A and/or B chain of ricin. The ricin gene has been cloned and sequenced, and the X-ray crystal structures of the A and B chains are published (Rutenber, E., et al. Proteins 10:240-250 (1991); Weston et al., *Mol. Biol.* 244:410-422

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(1994); Lamb and Lord, Eur. J. Biochem. 14:265 (1985); Halling, K., et al., Nucleic Acids Res. 13:8019 (1985)). It will be appreciated that the invention includes nucleic acid molecules encoding truncations of A and B chains of ricin like proteins and analogs and homologs of A and B chains of ricin-like proteins and truncations thereof (i.e., ricin-like proteins), as described herein. It will further be appreciated that variant forms of the nucleic acid molecules of the invention which arise by alternative splicing of an mRNA corresponding to a cDNA of the invention are encompassed by the invention.

10 Another aspect of the invention provides a nucleotide sequence which hybridizes under high stringency conditions to a nucleotide sequence encoding the A and/or B chains of a ricin-like Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or can be found in 15 Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1 6.3.6. For example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C may be employed. The stringency may be selected based on the conditions used in the wash step. By way of example, the salt concentration in the wash step 20 can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65°C.

The nucleic acid molecule may comprise the A and/or B chain of a ricin-like toxin. Methods for cloning ricin-like toxins are known in the art and are described, for example, in E.P. 466,222. Sequences encoding ricin or ricin-like A and B chains may be obtained by selective amplification of a coding region, using sets of degenerative primers or probes for selectively amplifying the coding region in a genomic or cDNA library. Appropriate primers may be selected from the nucleic acid sequence of A and B chains of ricin or ricin-like toxins. It is also possible to design synthetic oligonucleotide primers from the nucleotide sequences for use in PCR. Suitable primers may be selected

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from the sequences encoding regions of ricin-like proteins which are highly conserved, as described for example in U.S. Patent No 5,101,025 and E.P. 466,222.

A nucleic acid can be amplified from cDNA or genomic DNA using these oligonucleotide primers and standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. It will be appreciated that cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., Biochemistry 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL). It will be appreciated that the methods described above may be used to obtain the coding sequence from plants, bacteria or fungi, preferably plants, which produce known ricin-like proteins and also to screen for the presence of genes encoding as yet unknown ricin-like proteins.

A sequence containing a cleavage recognition site for a specific protease may be selected based on the disease or the pathogen which is to be targeted by the recombinant protein. The cleavage recognition site may be selected from sequences known to encode a cleavage recognition site for the cancer, viral or parasitic protease. Sequences encoding cleavage recognition sites may be identified by testing the expression product of the sequence for susceptibility to cleavage by the respective protease.

A sequence containing a cleavage recognition site for a viral, fungal, parasitic or cancer associated protease may be selected based on the retrovirus which is to be targeted by the recombinant protein. The cleavage recognition site may be selected from sequences known to encode a cleavage recognition site for the viral, fungal,

parasitic or cancer associated protease. Sequences encoding cleavage recognition sites may be identified by testing the expression product of the sequence for susceptibility to cleavage by a viral, fungal, parasitic or cancer associated protease. A polypeptide containing the suspected cleavage recognition site may be incubated with a protease and the amount of cleavage product determined (Dilannit, 1990, J. Biol. Chem. 285: 17345-17354 (1990)).

The protease may be prepared by methods known in the art and used to test suspected cleavage recognition sites.

In one embodiment, the preparation of tumour-associated cathepsin B, its substrates and enzymatic activity assay methodology have been described by Sloane, B.F. et al. (*Proc. Natl. Acad. Sci. USA* 83:2483-2487 (1986)), Schwartz, M.K. (*Clin. Chim. Acta* 237:67-78 (1995)), and Panchal, R.G. et al. (*Nature Biotechnol.* 14:852-856 (1996)).

The preparation of Epstein-Barr virus protease, its substrates and

The preparation of Epstein-Barr virus protease, its substrates and enzymatic activity assay methodology have been described by Welch, A.R. (*Proc. Natl. Acad. Sci. USA* 88:10792-10796 (1991)).

In another embodiment, the preparation of *Plasmodium* falciparum proteases, their substrates and enzymatic activity assay methodology have been described by Goldberg, D.E. et al. (*J. Exp. Med.* 173:961-969 (1991)), Cooper & Bujard (*Mol. Biochem. Parasitol.* 56:151-160 (1992)), Nwagwu, M. et al. (*Exp. Parasitol.* 75:399-414 (1992)), Rosenthal, P.J. et al. (*J. Clin. Invest.* 91:1052-1056 (1993)), Blackman, M.J. et al. (*Mol. Biochem. Parasitol.* 62:103-114 (1995)).

In a further embodiment, the preparation of proteases from human cytomegalovirus, human herpes virus, varicalla zoster virus and infectious laryngotracheitis virus have been taught by Liu F. & Roizman, B. (J. Virol. 65:5149-5156 (1991)) and Welch, A.R. (Proc. Natl. Acad. Sci. USA 88:10792-10796 (1991)). In addition, their respective substrates and enzymatic activity assay methodologies are also described.

In another embodiment, the preparation of hepatitis A virus protease, its substrates and enzymatic activity assay methodology have been described by Jewell, D.A. et al. (*Biochemistry* 31:7862-7869 (1992)). The preparation of poliovirus protease, its substrates and enzymatic activity assay methodology have been described by Weidner, J.R. et al. (*Arch. Biochem. Biophys.* 286:402-408 (1991)). The preparation of human rhinovirus protease, its substrates and enzymatic activity assay methodology have been described by Long, A.C. et al. (*FEBS Lett.* 258:75-78 (1989)).

In another embodiment of the invention, the preparation of proteases associated with Candida yeasts their substrates and enzymatic activity are contemplated, including the aspartic proteinases which have been associated specifically with numerous virulent strains of Candida including Candida albican, Candida tropicalis, and Candida parapsilosis (Abad-Zapatero, C. et al., Protein Sci. 5:640-652 (1996); Cutfield, S.M. et al., Biochemistry 35:398-410 (1995); Ruchel, R. et al, Zentralbl. Bakteriol. Mikrobiol Hyg. I Abt. Orig. A. 255:537-548 (1983); Remold, H. et al., Biochim. Biophys. Acta 167:399-406 (1968)).

The nucleic acid molecule of the invention may be prepared by site directed mutagenesis. For example, the cleavage site of a disease-specific protease may be prepared by site directed mutagenesis of the homologous linker sequence of a proricin-like toxin. Procedures for cloning proricin-like genes, encoding a linker sequence are described in EP 466,222. Site directed mutagenesis may be accomplished by DNA amplification of mutagenic primers in combination with flanking primers. Suitable procedures using the mutagenic primers are shown in Parts A and B of Figures 1-4, Figures 13-16, Figures 18-36, Figures 38-41, and Figures 50-67.

The nucleic acid molecule of the invention may also encode a fusion protein. A sequence encoding a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease may be cloned from a cDNA or genomic library or chemically

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synthesized based on the known sequence of such cleavage sites. The heterologous linker sequence may then be fused in frame with the sequences encoding the A and B chains of the ricin-like toxin for expression as a fusion protein. It will be appreciated that a nucleic acid molecule encoding a fusion protein may contain a sequence encoding an A chain and a B chain from the same ricin-like toxin or the encoded A and B chains may be from different toxins. For example, the A chain may be derived from ricin and the B chain may be derived from abrin. A protein may also be prepared by chemical conjugation of the A and B chains and linker sequence using conventional coupling agents for covalent attachment.

An isolated and purified nucleic acid molecule of the invention which is RNA can be isolated by cloning a cDNA encoding an A and B chain and a linker into an appropriate vector which allows for transcription of the cDNA to produce an RNA molecule which encodes a protein of the invention. For example, a cDNA can be cloned downstream of a bacteriophage promoter, (e.g. a T7 promoter) in a vector, cDNA can be transcribed in vitro with T7 polymerase, and the resultant RNA can be isolated by standard techniques.

#### 20 Recombinant Protein of the Invention

As previously mentioned, the invention provides novel recombinant proteins which incorporate the A and B chains of a ricin like toxin linked by a heterologous linker sequence containing a cleavage recognition site for a disease-specific protease. It is an advantage of the recombinant proteins of the invention that they are non-toxic until the A chain is liberated from the B chain by specific cleavage of the linker by the target protease.

Thus the protein may be used to specifically target cancer cells or cells infected with a virus or parasite in the absence of additional specific cell-binding components to target infected cells. It is a further advantage that the disease-specific protease cleaves the heterologous linker intracellularly thereby releasing the toxic A chain directly into

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the cytoplasm of the cancer cell or infected cell. As a result, said cells are specifically targeted and non-infected normal cells are not directly exposed to the activated free A chain.

Ricin is a plant derived ribosome inhibiting protein which blocks protein synthesis in eukaryotic cells. Ricin may be derived from the seeds of *Ricinus communis* (castor oil plant). The ricin toxin is a glycosylated heterodimer with A and B chain molecular masses of 30,625 Da and 31,431 Da respectively. The A chain of ricin has an N-glycosidase activity and catalyzes the excision of a specific adenine residue from the 28S rRNA of eukaryotic ribosomes (Endo, Y; & Tsurugi, K. J. Biol. Chem. 262:8128 (1987)). The B chain of ricin, although not toxic in itself, promotes the toxicity of the A chain by binding to galactose residues on the surface of eukaryotic cells and stimulating receptor-mediated endocytosis of the toxin molecule (Simmons et al., *Biol. Chem.* 261:7912 (1986)).

All protein toxins are initially produced in an inactive, precursor form. Ricin is initially produced as a single polypeptide (preproricin) with a 35 amino acid N-terminal presequence and 12 amino acid linker between the A and B chains. The pre-sequence is removed during translocation of the ricin precursor into the 20 endoplasmic reticulum (Lord, J.M., Eur. J. Biochem. 146:403-409 (1985) and Lord, J.M., Eur. J. Biochem. 146:411-416 (1985)). The proricin is then translocated into specialized organelles called protein bodies where a plant protease cleaves the protein at a linker region between the A and 25 B chains (Lord, J.M. et al., FASAB Journal 8:201-208 (1994)). The two chains, however, remain covalently attached by an interchain disulfide bond (cysteine 259 in the A chain to cysteine 4 in the B chain) and mature disulfide linked ricin is stored in protein bodies inside plant cells. The A chain is inactive in the proricin (O'Hare, M., et al., FEBS Lett. 273:200-204 (1990)) and it is inactive in the disulfide-linked mature 30 ricin (Richardson, P.T. et al., FEBS Lett. 255:15-20 (1989)). The ribosomes of the castor bean plant are themselves susceptible to inactivation by

ricin A chain; however, as there is no cell surface galactose to permit B chain recognition the A chain cannot re-enter the cell.

Ricin-like proteins include, but are not limited to, bacterial, fungal and plant toxins which have A and B chains and inactivate ribosomes and inhibit protein synthesis. The A chain is an active polypeptide subunit which is responsible for the pharmacologic effect of the toxin. In most cases the active component of the A chain is an enzyme. The B chain is responsible for binding the toxin to the cell surface and is thought to facilitate entry of the A chain into the cell cytoplasm. The A and B chains in the mature toxins are linked by disulfide bonds. The toxins most similar in structure to ricin are plant toxins which have one A chain and one B chain. Examples of such toxins include abrin which may be isolated from the seeds of Abrus precatorius and modeccin.

Ricin-like bacterial proteins include diphtheria toxin, which is produced by Corynebacterium diphtheriae, *Pseudomonas* enterotoxin A and cholera toxin. It will be appreciated that the term ricin-like toxins is also intended to include the A chain of those toxins which have only an A chain. The recombinant proteins of the invention could include the A chain of these toxins conjugated to, or expressed as, a recombinant protein with the B chain of another toxin. Examples of plant toxins having only an A chain include trichosanthin, MMC and pokeweed antiviral proteins, dianthin 30, dianthin 32, crotin II, curcin II and wheat germ inhibitor. Examples of fungal toxins having only an A chain include alpha-sarcin, restrictocin, mitogillin, enomycin, phenomycin. Examples of bacterial toxins having only an A chain include cytotoxin from Shigella dysenteriae and related Shiga-like toxins. Recombinant trichosanthin and the coding sequence thereof is disclosed in U.S. Patents 5,101,025 and 5,128,460.

In addition to the entire A or B chains of a ricin-like toxin, it will be appreciated that the recombinant protein of the invention may contain only that portion of the A chain which is

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necessary for exerting its cytotoxic effect. For example, the first 30 amino acids of the ricin A chain may be removed resulting in a truncated A chain which retains toxic activity. The truncated ricin or ricin-like A chain may be prepared by expression of a truncated gene or by proteolytic degradation, for example with Nagarase (Funmatsu et al., *Jap. J. Med. Sci. Biol.* 23:264-267 (1970)). Similarly, the recombinant protein of the invention may contain only that portion of the B chain necessary for galactose recognition, cell binding and transport into the cell cytoplasm. Truncated B chains are described for example in E.P. 145,111. The A and B chains may be glycosylated or non-glycosylated. Glycosylated A and B chains may be obtained by expression in the appropriate host cell capable of glycosylation. Non-glycosylated chains may be obtained by expression in nonglycosylating host cells or by treatment to remove or destroy the carbohydrate moieties.

The proteins of the invention may be prepared using recombinant DNA methods. Accordingly, the nucleic acid molecules of the present invention may be incorporated in a known manner into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression vectors are "suitable for transformation of a host cell", which means that the expression vectors contain a nucleic acid molecule of the invention and regulatory sequences selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid molecule. Operatively linked is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid.

The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory

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sequences for the transcription and translation of the inserted proteinsequence.

Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes (For example, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Selection of appropriate regulatory sequences is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. It will also be appreciated that the necessary regulatory sequences may be supplied by the native A and B chains and/or its flanking regions.

The recombinant expression vectors of the invention may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, β-galactosidase, chloramphenicol acetyltransferase, firefly luciferase, or an immunoglobulin or portion thereof such as the Fc portion of an immunoglobulin preferably IgG. Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as β-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have

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incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of recombinant expression vectors of the invention and in particular to determine the effect of a mutation on expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes which encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid in the purification of the target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the recombinant protein.

Recombinant expression vectors can be introduced into host cells to produce a transformant host cell. The term "transformant host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran mediated transfection, lipofectin, electroporation or microinjection.

Suitable methods for transforming and transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks.

Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991).

More particularly, bacterial host cells suitable for carrying out the present invention include E. coli, B. subtilis, Salmonella typhimurium, and various species within the genus' Pseudomonas, Streptomyces, and Staphylococcus, as well as many other bacterial species well known to one of ordinary skill in the art. Suitable bacterial expression vectors preferably comprise a promoter which functions in the host cell, one or more selectable phenotypic markers, and a bacterial origin of replication. Representative promoters include the β-lactamase (penicillinase) and lactose promoter system (see Chang et al., Nature 275:615 (1978)), the trp promoter (Nichols and Yanofsky, Meth in Enzymology 101:155, (1983) and the tac promoter (Russell et al., Gene 20: 231, (1982)). Representative selectable markers include various antibiotic resistance markers such as the kanamycin or ampicillin resistance genes. Suitable expression vectors include but are not limited to bacteriophages such as lambda derivatives or plasmids such as pBR322 (Bolivar et al., Gene 2:9S, (1977)), the pUC plasmids pUC18, pUC19, pUC118, pUC119 (see Messing, Meth in Enzymology 101:20-77, 1983 and Vieira and Messing, Gene 19:259-268 (1982)), and pNH8A, pNH16a, pNH18a, and Bluescript M13 (Stratagene, La Jolla, Calif.). Typical fusion expression vectors which may be used are discussed above, e.g. pGEX (Amrad Corp., Melbourne, Australia), pMAL (New

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England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ). Examples of inducible non-fusion expression vectors include pTrc (Amann et al., *Gene* 69:301-315 (1988)) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California, 60-89 (1990)).

Yeast and fungi host cells suitable for carrying out the present invention include, but are not limited to Saccharomyces cerevisae, the genera Pichia or Kluyveromyces and various species of the genus Aspergillus. Examples of vectors for expression in yeast S. cerivisae include pYepSec1 (Baldari. et al., Embo J. 6:229-234 (1987)), pMFa (Kurjan and Herskowitz, Cell 30:933-943 (1982)), pJRY88 (Schultz et al., Gene 54:113-123 (1987)), and pYES2 (Invitrogen Corporation, San Diego, CA). Protocols for the transformation of yeast and fungi are well known to those of ordinary skill in the art.(see Hinnen et al., Proc. Natl. Acad. Sci. USA 75:1929 (1978); Itoh et al., J. Bacteriology 153:163 (1983), and Cullen et al. (Bio/Technology 5:369 (1987)).

Mammalian cells suitable for carrying out the present invention include, among others: COS (e.g., ATCC No. CRL 1650 or 1651), BHK (e.g. ATCC No. CRL 6281), CHO (ATCC No. CCL 61), HeLa (e.g., ATCC No. CCL 2), 293 (ATCC No. 1573) and NS-1 cells. Suitable expression vectors for directing expression in mammalian cells generally include a promoter (e.g., derived from viral material such as polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40), as well as other transcriptional and translational control sequences. Examples of mammalian expression vectors include pCDM8 (Seed, B., *Nature* 329:840 (1987)) and pMT2PC (Kaufman et al., *EMBO J.* 6:187-195 (1987)).

Given the teachings provided herein, promoters, terminators, and methods for introducing expression vectors of an appropriate type into plant, avian, and insect cells may also be readily accomplished. For example, within one embodiment, the proteins of the invention may be expressed from plant cells (see Sinkar et al., *J. Biosci* (Bangalore) 11:47-58 (1987), which reviews the use of

Agrobacterium rhizogenes vectors; see also Zambryski et al., Genetic Engineering, Principles and Methods, Hollaender and Setlow (eds.), Vol. VI, pp. 253-278, Plenum Press, New York (1984), which describes the use of expression vectors for plant cells, including, among others, pAS2022, pAS2023, and pAS2034).

Insect cells suitable for carrying out the present invention include cells and cell lines from *Bombyx*, *Trichoplusia* or *Spodotera* species. Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith et al., *Mol. Cell Biol.* 3:2156-2165 (1983)) and the pVL series (Lucklow, V.A., and Summers, M.D., *Virology* 170:31-39 (1989)). Some baculovirus-insect cell expression systems suitable for expression of the recombinant proteins of the invention are described in PCT/US/02442.

Alternatively, the proteins of the invention may also be expressed in non-human transgenic animals such as, rats, rabbits, sheep and pigs (Hammer et al. *Nature* 315:680-683 (1985); Palmiter et al. *Science* 222:809-814 (1983); Brinster et al. *Proc. Natl. Acad. Sci. USA* 82:4438-4442 (1985); Palmiter and Brinster *Cell* 41:343-345 (1985) and U.S. Patent No. 4,736,866).

The proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, *J. Am. Chem. Assoc.* 85:2149-2154 (1964)) or synthesis in homogenous solution (Houbenweyl, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart (1987)).

The present invention also provides proteins comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for a disease-specific protease. Such a protein could be prepared other than by recombinant means, for example by chemical synthesis or by conjugation of A and B chains and a linker sequence isolated and

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purified from their natural plant, fungal or bacterial source. Such A and B chains could be prepared having the glycosylation pattern of the native ricin-like toxin.

N-terminal or C-terminal fusion proteins comprising the protein of the invention conjugated with other molecules, such as proteins may be prepared by fusing, through recombinant techniques. The resultant fusion proteins contain a protein of the invention fused to the selected protein or marker protein as described herein. The recombinant protein of the invention may also be conjugated to other proteins by known techniques. For example, the proteins may be coupled using heterobifunctional thiol-containing linkers as described in WO 90/10457, N-succinimidyl-3-(2-pyridyldithio-proprionate) or N-succinimidyl-5 thioacetate. Examples of proteins which may be used to prepare fusion proteins or conjugates include cell binding proteins such as immunoglobulins, hormones, growth factors, lectins, insulin, low density lipoprotein, glucagon, endorphins, transferrin, bombesin, asialoglycoprotein glutathione-S-transferase (GST), hemagglutinin (HA), and truncated myc.

# Utility of the Nucleic Acid Molecules and Proteins of the Invention

20 The proteins of the invention may be used to specifically inhibit or destroy mammalian cells affected by a disease or infection which have associated with such cells a specific protease, i.e., diseasespecific, for example cancer cells or cells infected with a virus, fungus or parasite, all of which are encompased within the term "disease-specific." It is an advantage of the recombinant proteins of the invention that 25 they have specificity for said cells without the need for a cell binding The ricin-like B chain of the recombinant proteins recognize galactose moieties on the cell surface and ensure that the protein is taken up by the diseased cell and released into the cytoplasm. When the protein is internalized into a non-infected cell, cleavage of 30 the heterologous linker would not occur in the absence of the diseasespecific protease and the A chain will remain inactive bound to the B

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chain. Conversely, when the protein is internalized into a diseased cell, the disease-specific protease will cleave the cleavage recognition site in the linker thereby releasing the toxic A chain.

The specificity of a recombinant protein of the invention may be tested by treating the protein with the disease-specific protease which is thought to be specific for the cleavage recognition site of the linker and assaying for cleavage products. Disease-specific proteases may be isolated from cancer cells or infected cells, or they may be prepared recombinantly, for example following the procedures in Darket et al. (J. Biol. Chem. 254:2307-2312 (1988)). The cleavage products may be identified for example based on size, antigenicity or activity. The toxicity of the recombinant protein may be investigated by subjecting the cleavage products to an in vitro translation assay in cell lysates, for example using Brome Mosaic Virus mRNA as a template. Toxicity of the cleavage products may be determined using a ribosomal inactivation assay (Westby et al., Bioconjugate Chem. 3:377-382 (1992)). The effect of the cleavage products on protein synthesis may be measured in standardized assays of in vitro translation utilizing partially defined cell free systems composed for example of a reticulocyte lysate preparation as a source of ribosomes and various essential cofactors, such as mRNA template and amino acids. Use of radiolabelled amino acids in the mixture allows quantitation of incorporation of free amino acid precursors into trichloroacetic acid precipitable proteins. Rabbit reticulocyte lysates may be conveniently used (O'Hare, FEBS Lett. 273:200-204 (1990)).

The ability of the recombinant proteins of the invention to selectively inhibit or destroy animal cancer cells or cells infected with a virus or parasite may be readily tested *in vitro* using animal cancer cell lines or cell cultures infected with the virus or parasite of interest. The selective inhibitory effect of the recombinant proteins of the invention may be determined, for example, by demonstrating the selective inhibition of viral antigen expression in infected mammalian

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cells, the selective inhibition of general mRNA translation and protein synthesis in diseased cells, or selective inhibition of cellular proliferation in cancer cells or infected cells.

Toxicity may also be measured based on cell viability, for example the viability of infected and non-infected cell cultures exposed to the recombinant protein may be compared. Cell viability may be assessed by known techniques, such as trypan blue exclusion assays.

In another example, a number of models may be used to test the cytotoxicity of recombinant proteins having a heterologous linker sequence containing a cleavage recognition site for a cancerassociated matrix metalloprotease. Thompson, E.W. et al. (Breast Cancer Res. Treatment 31:357-370 (1994)) has described a model for the determination of invasiveness of human breast cancer cells in vitro by measuring tumour cell-mediated proteolysis of extracellular matrix and tumour cell invasion of reconstituted basement membrane (collagen, laminin, fibronectin, Matrigel or gelatin). Other applicable cancer cell models include cultured ovarian adenocarcinoma cells (Young, T.N. et al. Gynecol. Oncol. 62:89-99 (1996); Moore, D.H. et al. Gynecol. Oncol. 65:78-82 (1997)), human follicular thyroid cancer cells (Demeure, M.J. et al., World J. Surg. 16:770-776 (1992)), human melanoma (A-2058) and fibrosarcoma (HT-1080) cell lines (Mackay, A.R. et al. Lab. Invest. 70:781-783 (1994)), and lung squamous (HS-24) and adenocarcinoma (SB-3) cell lines (Spiess, E. et al. J. Histochem. Cytochem. 42:917-929 (1994)). An in vivo test system involving the implantation of tumours and measurement of tumour growth and metastasis in athymic nude mice has also been described (Thompson, E.W. et al., Breast Cancer Res. Treatment 31:357-370 (1994); Shi, Y.E. et al., Cancer Res. 53:1409-1415 (1993)).

A further model may be used to test the cytotoxicity of 30 recombinant proteins having a heterologous linker sequence containing a cleavage recognition site for a cancer-associated Cathepsin WO 98/49311 PCT/CA98/00394

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B protease is provided in human glioma (Mikkelsen, T. et al. J. Neurosurge, 83:285-290 (1995)).

Similarly, the cytotoxicity of recombinant proteins having a heterologous linker sequence containing a cleavage recognition site for a malarial protease may be tested by a Plasmodium invasion assay using human erythrocytes infected with mature-stage merozoite parasites as described by McPherson, R.A. et al. (*Mol. Biochem. Parasitol.* 62:233-242 (1993)). Alternatively, in vitro cultures of human hepatic parenchymal cells may be used to evaluate schizont infectivity and Plasmodium merozoite generation.

With respect to models of viral infection and replication, suitable animal cells which can be cultured in vitro and which are capable of maintaining viral replication can be used as hosts. toxicity of the recombinant protein for infected and non-infected cultures may then be compared. The ability of the recombinant protein of the invention to inhibit the expression of these viral antigens may be an important indicator of the ability of the protein to inhibit viral replication. Levels of these antigens may be measured in assays using labelled antibodies having specificity for the antigens. Inhibition of viral antigen expression has been correlated with inhibition of viral replication (U.S. Patent No. 4,869,903). Toxicity may also be assessed based on a decrease in protein synthesis in target cells, which may be measured by known techniques, such as incorporation of labelled amino acids, such as [3H] leucine (O'Hare et al., FEBS Lett. 273:200-204 (1990)). Infected cells may also be pulsed with radiolabelled thymidine and incorporation of the radioactive label into cellular DNA may be taken as a measure of cellular proliferation. Toxicity may also be measured based on cell death or lysis, for example, the viability of infected and non-infected cell cultures exposed to the recombinant protein may be compared. Cell viability may be assessed by known techniques, such as trypan blue exclusion assays.

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Although the primary specificity of the proteins of the invention for diseased cells is mediated by the specific cleavage of the cleavage recognition site of the linker, it will be appreciated that specific cell binding components may optionally be conjugated to the proteins of the invention. Such cell binding components may be expressed as fusion proteins with the proteins of the invention or the cell binding component may be physically or chemically coupled to the protein component. Examples of suitable cell binding components include antibodies to cancer, viral or parasitic proteins.

Antibodies having specificity for a cell surface protein may be prepared by conventional methods. A mammal, (e.g. a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the peptide which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g. the hybridoma technique originally developed by Kohler and Milstein (Nature 256:495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol. Today 4:72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., Monoclonal Antibodies in Cancer Therapy Allen R., Bliss,

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Inc., pages 77-96 (1985)), and screening of combinatorial antibody libraries (Huse et al., *Science* 246:1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated.

The term "antibody" as used herein is intended to include fragments thereof which also specifically react with a cell surface component. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above. For example, F(ab')2 fragments can be generated by treating antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region are also contemplated within the scope of the invention. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. Conventional methods may be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes a cell surface antigen (See, for example, Morrison et al., *Proc. Natl Acad. Sci. U.S.A.* 81:6851 (1985); Takeda et al., *Nature* 314:452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., E.P. Patent No. 171,496; European Patent No. 173,494, United Kingdom Patent No. GB 2177096B). It is expected that chimeric antibodies would be less immunogenic in a human subject than the corresponding non-chimeric antibody.

Monoclonal or chimeric antibodies specifically reactive against cell surface components can be further humanized by producing human constant region chimeras, in which parts of the variable regions, particularly the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such

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immunoglobulin molecules may be made by techniques known in the art, (e.g. Teng et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80:7308-7312 (1983); Kozbor et al., *Immunology Today* 4:7279 (1983); Olsson et al., *Meth. Enzymol.*, 92:3-16 (1982), and PCT Publication WO92/06193 or EP 239,400). Humanized antibodies can also be commercially produced (Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.)

Specific antibodies, or antibody fragments, reactive against cell surface components may also be generated by screening expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with cell surface components. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries (See for example Ward et al., *Nature* 341:544-546 (1989); Huse et al., *Science* 246:1275-1281 (1989); and McCafferty et al., *Nature* 348:552-554 (1990)). Alternatively, a SCID-hu mouse, for example the model developed by Genpharm, can be used to produce antibodies, or fragments thereof.

The proteins of the invention may be formulated into pharmaceutical compositions for adminstration to subjects in a biologically compatible form suitable for administration in vivo. By "biologically compatible form suitable for administration in vivo" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to living organisms including humans, and animals. Administration of a therapeutically active amount of the pharmaceutical compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be

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proportionally reduced as indicated by the exigencies of the therapeutic situation.

The nucleic acid molecules of the invention may be formulated into pharmaceutical compositions for adminstration to subjects in a biologically compatible form suitable for administration in vivo. By "biologically compatible form suitable for administration in vivo" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to living organisms including humans, and animals. Administration of a therapeutically active amount of the pharmaceutical compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active substance may be administered in a convenient manner such as by injection (subcutaneous, intravenous, intramuscular, etc.), oral administration, inhalation, transdermal administration (such as topical cream or ointment, etc.), or suppository applications. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with

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a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The pharmaceutical compositions may be used in methods for treating animals, including mammals, preferably humans, with cancer or infected with a virus or a parasite. It is anticipated that the compositions will be particularly useful for treating patients with B-cell lymphoproliferative disease, (melanoma), mononucleosis, cytomegalic inclusion disease, malaria, herpes, shingles, hepatitis, poliomyelitis, or infectious laryngotracheitis. The dosage and type of recombinant protein to be administered will depend on a variety of factors which may be readily monitored in human subjects. Such factors include the etiology and severity (grade and stage) of neoplasia, the stage of malarial infection (e.g. exoerythrocytic vs. erythrocytic), or antigen levels associated with viral load in patient tissues or circulation.

As mentioned above, the novel recombinant toxic proteins and nucleic acid molecules of the present invention are useful in treating cancerous or infected cells wherein the cells contain a specific protease that can cleave the linker region of the recombinant toxic protein. One skilled in the art can appreciate that many different recombinant toxic proteins can be prepared once a disease associated protease has been identified. For example, the novel recombinant toxic proteins and nucleic acid molecules of the invention may be used to treat CNS tumors. Muller et al. (1993) describe increased activity of Insulin-type Growth Factor Binding Protein-3 (IGFBP-3) protease in the Cerebral Spinal Fluid of patients with CNS tumors. Cohen et al. (1992) claim that prostate-specific antigen (PSA) is an IGFBP-3 protease. The

pAP290 construct described above is a substrate for PSA. Conover et al. (1994) claim that cathepsin D is IGFBP-3 protease. The pAP276 described herein is a substrate for cathepsin D. Another example of a specific use of the invention is treatment of human glioma which has been shown to produce cathepsin D (Mikkelsen, T. et al. *J. Neurosurge*, 83:285-290 (1995)). The pAP 214 and 272 define herein are substrates for cathepsin B.

In addition, the novel proteins and nucleic acid molecules of the present invention may be used to treat cystic fibrosis. Hansen et al. (1995) describe how CF airway disease is characterized by neutrophil-dominated chronic inflammation with an excess of uninhibited neutrophil elastase (NE). NE levels in CF sputum are 350 times higher than that found in normal sputum. The pAP294 described herein is a substrate for neutrophil elastase.

As well, the novel proteins and nucleic acid molecules of the present invention may also be used to treat multiple sclerosis. Bever Jr. et al. (1994) implicate cathepsin B (possibly from inflammatory cells of hematogenous origin) in the demyelination found in multiple sclerosis. pAPs 214 and 272 defined herein present substrates for cathepsin B.

The term "animal" as used herein includes all members of the animal kingdom including mammals, preferably humans.

The following non-limiting examples are illustrative of the present invention:

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#### **EXAMPLES**

#### Example 1

## Cloning and Expression of Proricin Variants Activated by Disease-Specific Proteases

#### Isolation of total RNA

The preproricin gene was cloned from new foliage of the castor bean plant. Total messenger RNA was isolated according to established procedures (Sambrook et al., Molecular Cloning: A Lab

Manual (Cold Spring Harbour Press, Cold Spring Harbour, (1989)) and cDNA generated using reverse transcriptase.

cDNA Synthesis:

Oligonucleotides, corresponding to the extreme 5' and 3' ends of the preproricin gene were synthesized and used to PCR amplify the gene. Using the cDNA sequence for preproricin (Lamb et al., Eur. J. Biochem., 145:266-270, 1985), several oligonucleotide primers were designed to flank the start and stop codons of the preproricin open reading frame. The oligonucleotides were synthesized using an Applied Biosystems Model 392 DNA/RNA Synthesizer. First strand cDNA synthesis was primed using the oligonucleotide Ricin1729C (Table 1). Three micrograms of total RNA was used as a template for oligo Ricin1729C primed synthesis of cDNA using Superscript II Reverse Transcriptase (BRL) following the manufacturer's protocol.

## 15 DNA Amplification and Cloning

The first strand cDNA synthesis reaction was used as template for DNA amplification by the polymerase chain reaction The preproricin cDNA was amplified using the upstream primer Ricin-99 and the downstream primer Ricin1729C with Vent DNA polymerase (New England Biolabs) using standard procedures (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989)). Amplification was carried out in a Biometra thermal cycler (TRIO-Thermalcycler) using the following cycling parameters: denaturation 95°C for 1 min., annealing 52°C for 1 min., and extension 72°C for 2 min., (33 cycles), followed by a final extension cycle at 72°C for 10 min. The 1846bp amplified product was fractionated on an agarose gel (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor Laboratory Press, 1989), and the DNA purified from the gel slice using Qiaex resin (Qiagen) following the manufacturer's protocol. The purified PCR fragment encoding the preproricin cDNA was then ligated (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second

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Edition, (Cold Spring Harbor Laboratory Press, 1989)) into an Eco RV-digested pBluescript II SK plasmid (Stratagene), and used to transform competent XL1-Blue cells (Stratagene). Positive clones were confirmed by restriction digestion of purified plasmid DNA. Plasmid DNA was extracted using a Qiaprep Spin Plasmid Miniprep Kit (Qiagen).

### **DNA Sequencing**

The cloned PCR product containing the putative preproricin gene was confirmed by DNA sequencing of the entire cDNA clone (pAP-144). Sequencing was performed using an Applied Biosystems 373A Automated DNA Sequencer, and confirmed by double-stranded dideoxy sequencing by the Sanger method using the Sequenase kit (USB). The oligonucleotide primers used for sequencing were as follows: Ricin267, Ricin486, Ricin725, Ricin937, Ricin1151, Ricini1399, Ricin1627, T3 primer

- (5'AATTAACCCTCACTAAAGGG-3') (SEQ ID NO. 128) and T7 primer (5'GTAATACGACTCACTATAGGGC-3) (SEQ ID NO. 129). Sequence data was compiled and analyzed using PC Gene software package (intelligenetics). The sequences and location of oligonucleotide primers is shown in Table 1. The oligonucleotide primers shown in Table 1
- have been assigned the following sequence ID numbers:
  Ricin-109 is referred to herein as SEQ ID NO. 130;
  Ricin-99Eco is referred to herein as SEQ ID NO. 131;
  Ricin267 is referred to herein as SEQ ID NO. 132;
  Ricin486 is referred to herein as SEQ ID NO. 133;
- Ricin 725 is referred to herein as SEQ ID NO. 134;
  Ricin 937 is referred to herein as SEQ ID NO. 135;
  Ricin 1151 is referred to herein as SEQ ID NO. 136;
  Ricin 1399 is referred to herein as SEQ ID NO. 137;
  Ricin 1627 is referred to herein as SEQ ID NO. 138;
- 30 Ricin 1729C is referred to herein as SEQ ID NO. 139; and Ricin 1729C Xba is referred to herein as SEQ ID NO. 140.

  Production and Cloning of Linker Variants

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pAP144 cut with EcoRI was used as target for PCR pairs employing the Ricin109-Eco oligonucleotide (Ricin-109Eco primer: 5-GGAGGAATCCGGAGATGAAACCGGGAGGAAATACTATTGTAAT-3 (SEQ ID No. 141)) and a mutagenic primer for the 5' half of the linker as well as the Ricin1729PstI primer (Ricin1729-PstI: 5-GTAGGCGCTGCAGATAACTTGCTGTCCTTTCAG-3 (SEQ ID No. 142)) and a mutagenic primer for the 3' half of the linker. The cycling conditions used for the PCRs were 98 degrees C for 2 min.; 98C 1 min., 52C 1 min., 72C 1 min. 15 sec. (30 cycles); 72 degrees C 10min.; 4 degrees C soak. The PCR products were then digested by EcoRI and PstI respectively, electrophoresed on an agarose gel, and the bands purified by via glass wool spin columns. Triple ligations comprising the PCR product pairs (corresponding halves of the new linker) and pVL1393 vector digested with EcoRI and PstI were carried out. Recombinant clones were identified by restriction digests of plasmid miniprep DNA and the altered linkers confirmed by DNA sequencing. See Figure 45 as an example of the cloning strategy. Recombinant clones were identified by restriction digests of plasmid miniprep DNA and the altered linkers confirmed by DNA sequencing. Note that since all altered linker variants were cloned directly into the pVL1393 vector odd-numbered pAPs were no longer required or produced.

## Isolation of Recombinant Baculoviruses

Insect cells S. frugiperda (Sf9), and Trichoplusia ni (Tn368 and BTI-TN-581-4 (High Five)) were maintained on EX-CELL 405 medium (JRH Biosciences) supplemented with 10% total calf serum (Summers et al., A Manual of Methods of Baculovirus Vectors and Insect Cell Culture Procedures, (Texas Agricultural Experiment Station, 1987)). Two micrograms of recombinant pVL1393 DNA was cotransfected with 0.5 microgram of BaculoGold AcNPV DNA (Pharmingen) into 2 x 106 Tn368 insect cells following the manufacturer's protocol (Gruenwald et al., Baculovirus Expression Vector System: Procedures and Methods Manual, 2nd Edition, (San

Diego, CA, 1993)). On day 5 post-transfection, media were centrifuged and the supernatants tested in limiting dilution assays with Tn368 cells (Summers et al., A Manual of Methods of Baculovirus Vectors and Insect Cell Culture Procedures, (Texas Agricultural Experiment Station, 1987)). Recombinant viruses in the supernatants were then amplified by infecting Tn368 cells at a multiplicity of infection (moi) of 0.1, followed by collection of day 3 to 5 supernatants. A total of three rounds of amplification were performed for each recombinant following established procedures (Summers et al., A Manual of Methods of Baculovirus Vectors and Insect Cell Culture Procedures, (Texas Agricultural Experiment Station, 1987 and Gruenwald et al., Baculovirus Expression Vector System: Procedures and Methods Manual, 2nd Edition, (San Diego, CA, 1993)).

## **Expression of Mutant Proricin**

Recombinant baculoviruses were used to infect  $1X10^7$  Tn368 or sf9 cells at an moi of 9 in EX-CELL 405 media (JRH Biosciences) with 25mM  $\alpha$ -lactose in spinner flasks. Media supernatants containing mutant provicins were collected 3 or 4 days post-infection.

#### **EXAMPLE 2**

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## 20 Harvesting and affinity column purification of pro-ricin variants

Protein samples were harvested three days post transfection. The cells were removed by centrifuging the media at 8288 g for ten minutesusing a GS3 (Sorvall) centrifuge rotor. The supernatant was further clarified by centrifuging at 25400 g using a SLA-1500 rotor (Sorvall) for 45 minutes. Protease inhibitor phenylmethylsulfonyl fluoride (Sigma) was slowly added to a final concentration of 1mM. The samples were further prepared by adding lactose to a concentration of 20 mM (not including the previous lactose contained in the expression medium). The samples were concentrated to 700 mL using a Prep/Scale-TFF Cartridge (2.5ft, 10K regenerated cellulose (Millipore)) and a Masterflex pump. The samples were then

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dialysed for 2 days in 1X Column Buffer (50 mM Tris, 100 mM NaCl, 0.02% NaN3, pH 7.5) using dialysis tubing (10 K MWCO, 32 mm flat width(Spectra/Por)). Subsequently, the samples were clarified by centri fuging at 25400 g using a SLA-1500 rotor (Sorvall) for 45 minutes.

Following centrifugation, the samples were degassed and applied at 4 degrees C to a XK26/20 (Pharmacia) column (attached to a Pharmacia peristaltic pump, Pharmacia Single-path Monitor UV-1 Control and Optical Units, and Bromma LKB 2210 2-Channel Recorder) containing 20 mL of a-Lactose Agarose Resin (Sigma). The column was washed for 3 hours with 1X Column buffer. Elution of pro-ricin variant was performed by eluting with buffer (1X Column buffer (0.1% NaN3), 100 mM Lactose) until the baseline was again restored. The samples were concentrated using an Amicon 8050 concentrator (Amicon) with a YM10 76 mm membrane, utilizing argon gas to pressurize the chamber. The samples were further concentrated in Centricon 10 (Millipore) concentrators according to manufacturer's specifications.

## Purification of Variant pAP-Protein by gel filtration chromatography

In order to purify the pro-ricin variant from processed material produced during fermentation, the protein was applied to a SUPERDEX 75 (16/60) column and SUPERDEX 200 (16/60) column (Pharmacia) connected in series equilibrated with 50 mM Tris, 100mM NaCl, pH 7.5 containing 100 mM Lactose and 0.1%  $\beta$ -mercaptoethanol ( $\beta$ ME). The flow rate of the column was 0.15 mL/min and fractions were collected every 25 minutes. The UV (280 nm) trace was used to determine the approximate location of the purified pAP-protein and thus determine the samples for Western analysis.

## Western analysis of column fractions

Fractions eluted from the SUPERDEX columns (Pharmacia) were analyzed for purity using standard Western blotting techniques. An aliquot of 10μL from each fraction was boiled in 1X sample buffer (62.6 mM Tris-C1, pH 6.8, 4.4% βME, 2% sodium dodecyl

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sulfate (SDS), 5% glycerol (all from Sigma) and 0.002% bromophenol blue (Biorad)) for five minutes. Denatured samples were loaded on 12% Tris-Glycine Gels (Biorad) along with 50 ng of RCA $_{60}$  (Sigma) and 5  $\mu$ L of kaleidoscope prestained standards (Biorad). Electrophoresis was carried out for ninety minutes at 100V in 25 mM Tris-Cl, pH 8.3, 0.1% SDS, and 192 mM glycine using the BioRad Mini Protean II cells (Biorad).

Following electrophoresis gels were equilibrated in transfer buffer (48 mM Tris, 39 mM glycine, 0.0375% SDS, and 20% Methanol) for a few minutes. PVDF Biorad membrane was presoaked for one minute in 100% methanol, rinsed in ddH<sub>2</sub>O and two minutes in transfer buffer. Whatman paper was soaked briefly in transfer buffer. Five pieces of Whatman paper, membrane, gel, and another five pieces of Whatman paper were arranged on the bottom cathode (anode) of the Pharmacia Novablot transfer apparatus (Pharmacia). Transfer was for one hour at constant current (2 mA/cm<sup>2</sup>).

Transfer was confirmed by checking for the appearance of the prestained standards on the membrane. Non-specific sites on the membrane were blocked by incubating the blot for thirty minutes in 1X Phosphate Buffered Saline (1X PBS; 137 mM NaCl, 2.7 mM KCl, 8 mM 20 Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) with 5% skim milk powder (Carnation). Primary antibody (Rabbit  $\alpha$ -ricin, Sigma) was diluted 1:3000 in 1X PBS containing 0.1% Tween 20 (Sigma) and 2.5% skim milk and incubated with blot for forty five minutes on a orbital shaker (VWR). Non-specifically bound primary antibody was removed by washing the blot for ten minutes with 1X PBS containing 0.2% Tween 20. This was repeated four times. Secondary antibody donkey anti-rabbit (Amersham) was incubated with the blot under the same conditions as the primary antibody. Excess secondary antibody was washed as described above. Blots were developed with the ECL Western Blotting 30 detection reagents according to the manufacturer's instructions. Blots

were exposed to Medtec's Full Speed Blue Film (Medtee) or Amersham's ECL Hyperfilm (Amersham) for one second to five minutes. Film was developed in a KODAK Automatic Developer.

## Determination of lectin binding ability of pro-ricin variant

5 An Immulon 2 plate (VDVR) was coated with 100  $\mu l$  per well of 10µg/ml of asialofetuin and left overnight at 4°C. The plate was washed with 3X 300  $\mu$ L per well with ddH<sub>2</sub>O using an automated plate washer (BioRad). The plate was blocked for one hour at 37°C by adding 300 µL per well of PBS containing 1% ovalbumin. The plate was washed again as above. Pro-ricin variant pAP-protein was added to the 10 plate in various dilutions in 1X Baculo. A standard curve of RCA60 (Sigma) from 1-10 ng was also included. The plate was incubated for 1 h The plate was washed as above. Anti-ricin monoclonal antibody (Sigma) was diluted 1:3000 in 1X PBS containing 0.5% ovalbumin and 0.1% tween-20, added at 100  $\mu L$  per well and incubated 15 for 1 h at 37°C. The plate was washed as above. Donkey-anti rabbity polyclonal antibody was diluted 1:3000 in 1X PBS containing 0.5% ovalbumin, 0.1% Tween-20, and added at 100µL per well and incubated for 1 h at 37°C. The plate was given a final wash as described above. Substrate was added to plate at  $100\mu L$  per well (1 mg/ml o-20 phenylenediamine (Sigma), 1  $\mu$ L/ml  $H_2O_2$ , 25  $\mu$ L of stop solution (20% H<sub>2</sub>SO<sub>4</sub>) was added and the absorbance read (A490nm-A630nm) using a SPECTRA MAX 340 plate reader (Molecular Devices).

# Determination of pAP -Protein activity using the rabbit reticulocyte assay

Ricin samples were prepared for reduction.

A) RCA<sub>60</sub> = 3,500 ng/ $\mu$ L of RCA<sub>60</sub> + 997  $\mu$ L 1xEndo buffer (25mM Tris, 25mM KCl,5mM MGCl<sub>2</sub>, pH 7.6) Reduction = 95  $\mu$ L of 10ng/ $\mu$ L + 5  $\mu$ L  $\beta$ -mercaptoethanol

B) Ricin variants

Reduction =  $40 \mu L$  variant +  $2 \mu L$   $\beta$ -mercaptoethanol The ricin standard and the variants were incubated for 30 minutes at room temperature.

### 5 Ricin - Rabbit Reticulocyte lysate reaction

The required number of 0.5 mL tubes were labelled. (2) tubes for each sample, + and - aniline). To each of the sample tubes 20  $\mu L$  of 1X endo buffer was added, and 30  $\mu L$  of buffer was added to the controls. To the sample tubes either 10  $\mu L$  of 10ng/ $\mu L$  Ricin or 10 $\mu L$  of variant was added. Finally, 30 µL of rabbit reticulocyte lysate was added to all the tubes. The samples were incubated for 30 minutes at 30°C using the thermal block. Samples were removed from the eppendorf tube and contents added into a 1.5 mL tube containing 1 mL of TRIZOL (Gibco). Samples were incubated for 15 minutes at room temperature. After the incubation, 200 µL of chloroform was added, and the sample 15 was vortexed and spun at 12,000 g for 15 minutes at 4°C. The top aqueous layer from the samples was removed and contents added to a 1 mL tube containing 500  $\mu$ L of isopropanol. Samples were incubated for 15 minutes at room temperature and then centrifuged at 12,000 for 15 20 minutes at 4°C. Supernatant was removed and the pellets were washed with 1 mL of 70% ethanol. Centrifugation at 12,000 g for 5 minutes at 4°C precipitated the RNA. All but approximately 20 μL of the supernatant was removed and air dried. Pellets from the other samples (+aniline samples) were dissolved in 20  $\mu L$  of DEPC treated ddH<sub>2</sub>O. An  $80~\mu L$  aliquot of 1 M aniline (distilled) with 2.8 M acetic acid was added 25 to these RNA samples and transferred to a fresh 0.5 mL tube. The samples were incubated in the dark for 3 minutes at 60°C. RNA was precipitated by adding 100  $\mu L$  of 95% ethanol and  $5\mu L$  of 3M sodium acetate, pH 5.2 to each tube and centrifuging at 12,000 g for 30 minutes at

4°C. Pellets were washed with 1 mL 70% ethanol and centrifuged again at 12,000g for 5 minutes at 4°C to precipitate RNA. The supernatant was removed and air dried. These pellets were dissolved in 10μL of 0.1 X E buffer. To all samples, 10 μL of formamide loading dye was added. The RNA ladder (8 μL of ladder + 8 μL of loading dye) was also included. Samples were incubated for 2 minutes at 70°C on the thermal block. Electrophoresis was carried out on the samples using 1.2% agarose, 50% formamide gels in 0.1X E buffer + 0.2% SDS. The gel was run for 90 minutes at 75 watts. RNA was visualized by staining the gel in 1 μg/μL ethidium bromide in running buffer for 45 minutes. The gel was examined on a 302 nm UV box, photographed using the gel documentation system and saved to a computer disk.

#### **Results:**

### Protein Expression Yields

Aliquots were taken at each stop of the harvesting/purification and tested. Yields of functional ricin variant were determined by ELISA. Typical results of an 2400 mL prep of infected *T. ni* cells are given below.

	Aliquot µg pA	<u>µg pAP 220</u>	
20	Before concentration and dialysis	6000	
	After concentration and dialysis	4931	
	alpha- Lactose agarose column flow through	219	
	alpha- Lactose agarose column elution	1058	

25 Yield: 1058/6000 = 17.6%

## Purification of pAP -Protein and Western Analysis of column fractions

Partially purfied pAP-protein was applied to Superdex 75 and 200 (16/60) columns connected in series in order to remove the

contaminating non-specifically processed pAP-protein. Eluted fractions were tested via Western analysis as described above and the fractions containing the most pure protein were pooled, concentrated and reapplied to the column. The variant was applied a total of three times to the column. Final purified pAP-protein has less than 1% processed variant.

The purified pAP-protein was tested for susceptibility to cleavage by the particular protease and for activation of the A-chain of the proricin variant, (inhibition of protein synthesis). Typically, pAP-protein was incubated with and without protease for a specified time period and then electrophoresed and blotted. Cleaved pAP will run as two 30 kDa proteins (B is slightly larger) under reducing (SDS-PAGE) conditions. Unprocessed pAP-protein, which contains the linker region, will run at 60 kDa.

## 15 Activation of pAP -Protein variant with Specific Protease

Activation of protease treated pAP-protein is based on the method of *May et al.* (EMBO Journal. <u>8</u> 301-8, 1989). Activation of ricin A chain upon cleavage of the intermediary linker results in catalytic depurination of the adenosine 4325 residue of 28S or 26S rRNA. This depurination renders the molecule susceptible to amine-catalyzed hydrolysis by aniline of the phosphodiester bond on either side of the modification site. The result is a diagnostic 390 base band. As such, reticulocyte ribosomes incubated with biochemically purified ricin A chain, released the characteristic RNA fragment upon aniline treatment of isolated rRNA (May, M.J. et al. Embo. Journal, 8:301-308 at 302-303 (1989)). It is on this basis that the assay allows for the determination of activity of a ricin A chain which has been cleaved from the intact unit containing a particular variant linker sequence.

#### **EXAMPLE 3**

### 30 In Vitro Protease Digestion of Proricin Variants:

Affinity-purified proricin variant is treated with individual disease-specific proteases to confirm specific cleavage in the linker

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region. Ricin-like toxin variants are eluted from the lactose-agarose matrix in protease digestion buffer (50mM NaCl, 50mM Na-acetate, pH 5.5, 1mM dithiothreitol) containing 100mM lactose. Proricin substrate is then incubated at 37°C for 60 minutes with a disease-specific protease. The cleavage products consisting ricin A and B chains are identified using SDS/PAGE (Sambrook et al., Molecular Cloning: a Laboratory Manual, 2nd. ed., Cold Spring Harbor Press, 1989), followed by Western blot analysis using anti-ricin antibodies (Sigma).

Cathepsin B may be obtained from Medcor or Calbiochem. 10 Matrix metalloproteinases may be prepared substantially as described by Lark, M.W. et al. (Proceedings of the 4th International Conference of the Imflammation Research Association Abstract 145 (1988)) and Welch, A.R. et al. (Arch. Biochem. Biophys. 324:59-64 (1995)). Candida acid protease may be prepared substantially as described in Remold, H.H. et al. (Biochim. Biophys. Acta 167:399-406 (1968)), Ray, T.L. and Payne, C.D. 15 (Infect. Immunol. 58:508-514 (1990)) and Fusek, M. et al. (FEBS Lett. 327:108-112 (1993)). Hepatitis A protease may be prepared as described in Jewell, D.A. et al. (Biochemistry 31:7862-7869 (1992)). Plasmodium proteases may be prepared as described in Goldberg, D.E. et al. (J. Exp. Med. 173:961-969 (1991)) and Cooper, J.A. and Bujard, H. (Mol. Biochem. Parasitol. 56:151-160 (1992)).

## In Vitro Cytotoxicity Assay:

Human ovarian cancer cells (e.g. MA148) are seeded in 96-well flat-bottom plates and are exposed to ricin-like toxin variants or control medium at 37°C for 16 h. The viability of the cancer cells is determined by measuring [35S]methionine incorporation and is significantly lower in wells treated with the toxin variants than those with control medium.

## In Vivo Tumour Growth Inhibition Assay:

Human breast cancer (e.g. MCF-7) cells are maintained in suitable medium containing 10% fetal calf serum. The cells are grown, harvested and subsequently injected subcutaneously into

ovariectomized athymic nude mice. Tumour size is determined at intervals by measuring two right-angle measurements using calipers. In animals that received ricin-like toxin variants containing the matrix metalloproteinase-sensitive linkers, tumour size and the rate of tumour growth are lower than animals in the control group.

#### In Vivo Tumour Metastasis Assay:

The metastasis study is performed substantially as described in Honn, K.V. et al. (*Biochem. Pharmacol.* 34:235-241 (1985)). Viable B16a melanoma tumour cells are prepared and injected subcutaneously into the left axillary region of syngeneic mice. The extent of tumour metastasis is measured after 4 weeks. The lungs are removed from the animals and are fixed in Bouin's solution and macroscopic pulmonary metastases are counted using a dissecting microscope. In general without therapeutic intervention, injection of 10<sup>5</sup> viable tumour cells forms approximately 40-50 pulmonary metastases. The number of metastases in animal treated with proricin variants containing cathepsin B-sensitive linkers is substantially lower.

#### EXAMPLE 4

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# In Vitro Protease Digestion of Proricin Variants by Cancer Proteases 20 Cathepsin B or MMP-9

The general protocol for proricin digestion by cancer proteases is described in Examples 2 and 3.

## In Vitro Protease Digestion of Cathepsin B Proricin Variant

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region. The proricin substrate is digested in a Cathepsin B protease buffer (50 mM Sodium acetate, 2 mM EDTA, 0.05% Triton) at 40°C. Two hours and overnight (16 hr) digestion reactions are carried out using 100ng of proricin substrate and 100 and 618 ng of Cathepsin B protease per reaction (CALBIOCHEM, USA). The cleavage products of proricin (ricin A and B chains) are identified using SDS/PAGE (Sambrook et al., Molecular cloning: a laboratory Manual, 2nd. ed., Cold Spring Harbor

Press, 1989), followed by Western blot analysis using anti-ricin antibodies (Sigma).

## In Vitro Protease Digestion of MMP-9 Proricin Variant

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region. The proricin substrate is digested in 1X column buffer (100 mM NaCl, 50 mM Tris, PH 7.5) at 37°C. Two hours and overnight (16 hr) digestion reactions are set up using 50 ng of MMP-9 proricin substrate and 20 and 200 ng of MMP-9 protease per reaction (CALBIOCHEM, USA). The cleavage products of proricin (ricin A and B chains) are identified using SDS/PAGE (Sambrook et al., Molecular cloning: a laboratory Manual, 2nd. ed., Cold Spring Harbor Press, 1989), followed by Western blot analysis using anti-ricin antibodies (Sigma).

The protocol for Western analysis of ricin chains is described in Example 2.

#### Results

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Figures 48 and 49 illustrate Western blots showing the cleavage of the protease-sensitive linkers by cathepsin B (pAP 214) and MMP-9 (pAP 220) respectively. Without protease digestion, the proricin variant appears as a single band at approximately 60 kDa (Lane B of Figure 48 and Lane A of Figure 49). Wild type ricin A chain and B chain appear as two disparate bands at approximately 30 kDa (Lane A of Figure 48 and Lane E of Figure 49). Increasing extent of proricin cleavage can clearly be observed with increasing protease concentration (Lanes C and D of Figure 48 and Lanes B-C of Figure 49).

#### **EXAMPLE 5**

In vitro protease digestion of various proricin variants by their corresponding proteases.

The general protocol for proricin digestion by coresponding proteases was as desribed in Examples 2 and 3 and should be considered in connection with the digestions described below.

# Cleavage of pAP-222 protein with the Matrix Metalloproteinase 2 (MMP-2)

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

The pAP-222 protein sample (1.0 ug) was digested with the MMP-2 protease (1.0 ug) overnight at 37° C. The total volume of the digestion reaction was 21.5 ul, and 0.250 ug of the reaction sample was loaded on a protein gel. The MMP-2 protease was purchased from Calbiochem-Novabiochem Corporation, USA.

# <u>Cleavage of pAP-248 protein with the Human Cytomegalovirus</u> (HCMV) protease

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

The pAP-248 protein sample (1.19 ug) was digested with the HCMV protease (1.13 ug) overnight at 37°C. The total volume of the digestion was 10.5 ul, and 0.279 ug of the reaction sample was loaded on a protein gel. The HCMV was purchased from BACHEM Bioscience Inc., USA.

# 20 Cleavage of pAP-256 protein with the Hepatitis A virus 3C (HAV 3C) protease

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

The pAP-256 protein sample (1.26 ug) was digested with the HAV 3C protease (5 ug) overnight at 37°C. The total volume of the digestion was 12.5 ul, and 0.302 ug of the digestion sample was loaded on a protein gel. The HAV 3C protease was a gift from Dr. G. Lawson from Bates Collage, Main, USA.

# 30 Cleavage of pAP-270 protein with the Matrix Metallopr teinase 2 (MMP-2)

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Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

The pAP-270 protein sample (0.120 ug) was digested with the MMP-2 protease (0.25 ug) overnight at 37° C. The total volume of the digestion reaction was 22.5 ul, and 0.106 ug of the reaction sample was loaded on a protein gel. The MMP-2 protease was purchased from Calbiochem-Novabiochem Corporation, USA.

## Cleavage of pAP-288 protein with tPA plasminogen tissue activator

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region. The pAP-288 protein sample (1.65 ug) was digested with the t-PA protease (0.5 ug) overnight at 37° C. The total volume of the digestion reaction was 55 ul, and 0.6 ug of the reaction sample was loaded on a protein gel. The t-PA was purchased from Sigma Chemical Co., USA.

## Cleavage of pAP-294 protein with human neutraphil elastase

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region.

The pAP-256 protein sample (0.6 ug) was digested with the Elastase protease (5 ug) at 25°C for one hour. The total volume of the digestion reaction was 52.5 ul, and 0.171 ug of the digestion sample was loaded on a protein gel. The Human Neutrophil Elastase protease was purchased from Cedarlane Laboratories Limited, Canada.

## Cleavage of pAP-296 protein with calpain

Affinity-purified mutant proricin is treated with individual disease-specific proteases to confirm specific cleavage in the linker region. The pAP-296 protein sample (2.05 ug) was digested with the Calpain protease (10 ug) overnight at 37° C. The total volume of the digestion reaction was 35 ul and 0.761 ug of the reaction sample was

loaded on a protein gel. The Calpain protease was purchased from Sigma Chemical Co., USA

#### **Results**

Figures 52, 54, 58 & 66(MMP-2), 60, 64 and 62 show the cleavage of proteases of linkers by HCMV, HAV 3C, MMP-2, t-PA, calpain, and human neutraphil elastase respectively. Without protease digestion, the proricin variants appear as a single band at approximately 60kDA (Lane A in connection with Figure 52; Lane B of Figure 54; Lane A of Figure 58; Lane B of Figure 60; and Lane C of Figure 62; lane B of Figure 64 and lane B of Figure 66). Wild type ricin chain A and B appear as two bands at approximately 30kDA (see for example Lanes C and D of Figure 52) proricin cleavage can clearly be obvserved with the appearance of 30kDA bands in connection with the protein which has been digested by the respective protease (see Lane B of Figure 52; Lane C of Figure 54; or Lane B of Figure 58 for examples).

#### **EXAMPLE 6**

# In Vitro Translation Assay (Activation by Cancer Proteases Cathepsin B or MMP-9

The general protocol for the rabbit retoculocyte lysate reaction to test the cytotoxicity of cancer protease-activiated proricin is described briefly in Example 3 and is described in more detail in Example 2.

#### **Results**

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Activation of pAP 214 and pAP 220 proricin variants by cathepsin B and MMP-9, based on the method of May et al. (EMBO J. 8:301-308, 1989), is illustrated in Figures 50 and 51 respectively. The appearance of the 390 base pair product (positive control) is observed in Lane F of Figure 50 and Lane G of Figure 51. This 390 base pair product is absent in the negative control lanes. Without cathepsin or MMP-9 activation, no or minimal N-glycosidase activity in the pAP 214 variant (Lanes H to L, Figure 50) or the pAP 220 variant (Lanes A to E, Figure 51) was observed. When the pAP 214 variant and the pAP 220 variant were activated by cathepsin or MMP-9 respectively, appearance of the 390 base

pair product was observed in a proricin concentration-dependent manner (Lanes A to E of Figure 50 and Lanes H to L of Figure 51). The present experimental series demonstrated the successful and selective activation of proricin variants by cancer-associated proteases.

#### 5 EXAMPLE 7

The general protocol for the rabbit retoculocyte lysate reaction is described briefly in Example 3 and is described in more detail in Example 2, all of which compliments the description below.

# Depurination of Rabbit Reticulocyte 28S Ribosomal RNA by Digested and Undigested Ricin Variants

Affinity-purified mutant proricin mutants which were previously digested with the disease-specific protease, were reduced with 5% 2-mercaptoethanol then diluted to 100ng, 14.2ng,2.0ng,291pg, and 41.7pg with 1 X ENDO buffer(25mM Tris pH 7.6, 25mM KCl, 5mM MgCl<sub>2</sub>) and incubated with rabbit reticulocyte lysate, untreated (Promega) for 30minutes at 30(C. To compare the digested with the undigested proricin variant, the proricin in digestion buffer (according to the specific digestion protocol) was treated in the same manner as the digested sample. As a positive and negative control, 10ng of ricin A chain and 1 X ENDO buffer consecutively, was incubated with rabbit reticulocyte lysate, untreated, for 30 min at 30°C.

## Aniline Cleavage of rRNA and Gel Fractionation

Total RNA was then extracted from reticulocyte lysate translation mixtures with Trizol reagent (Gibco-BRL) as per manufacturer's instructions. The RNA was incubated with 80ul of 1M aniline (distilled) with 2.8M acetic acid for 3 min at 60(C in the dark. Ethanol-precipitated RNA samples were dissolved in 20ul of 50% formamide, 0.1X E buffer (3.6mM Tris, 3mM NaH<sub>2</sub>PO<sub>4</sub>, 0.2mM EDTA), and 0.05% xylene cyanol. 10ul of this was heated to 70(C for 2 minutes, loaded and electrophoresed in 1.2% agarose, 0.1X E buffer, and 50% formamide gel with RNA running buffer (0.1 X E buffer, 0.2% SDS). Results

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Activation of pAP-248 proricin variant by HCMV; pAP-256 by HAV3C protease; pAP-270 by MMP-2 protease; pAP-288 by t-PA protease; pAP-294 by human neutrophil elastase; pAP-296 by calpain; and pAP-222 by MMP-2 is illustrated in Figures 52, 55, 59, 61, 63, 65, and 67 respectively. The appearance of the 390 base pair product (deposit of control) is obverved in lane L of Figures 53, 55, 61, 63, 65 and 67. The 390 base pair product is observed in lane A of Figures 59 (activation of pAP-270 by MMP-2). This 390 base pair product is absent in the negative control lanes. Without the specific protease activation, no or minimal activity is seen in the lanes which contained only the proricin variant without digestion (see lane A, B, C, D, and E of Figures 53, 55, 61, 63, 65, and 67). The same observation is made in connection with pAP-270 in Figure 59, however, the undigested lanes appear as H, I, J, K and L. When the variant was activated by its respective protease, there is an appearance of the 390 base pair product in a proricin concentrationdependent manner (see Lanes H, I, J, K and L of Figure 53, 55, 61, 63, 65, and 67 and Lanes A, B, C, D, and E of Figure 59). The present experimental series demonstrate the successful and selective activation of the identified proricin variants by selective corresponding proteases.

#### 20 **EXAMPLE 8**

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## <u>Procedure for Examining the Cytotoxicity of Ricin and Ricin Variants</u> <u>on the COS-1 Cell Line</u>

#### **Cell Preparation**

After washing with 1XPBS (0.137 M NaCl, 2.68 mM KCl, 8.10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>), cells in log phase growth were removed from plates with 1X trypsin/EDTA (Gibco/BRL). The cells were centrifuged at 1100 rpm for 3 min, resuspended in Dulbecco's Modified Eagle Medium containing 10%FBS and 1X pen/strep, and then counted using a haemocytometer. They were adjusted to a concentration of 5 X 10<sup>4</sup> cells•ml<sup>-1</sup>. One hundred microliters per well of cells was added to wells 2B - 2G through to wells 9B - 9G of a Falcon 96 well tissue culture plate. A separate 96 well tissue culture plate was

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used for each sample of Ricin or Ricin variant. The plates were incubated at  $37(C \text{ with } 5\% \text{ CO}_2 \text{ for } 24 \text{ hours}.$ 

#### **Toxin Preparation**

The Ricin and Ricin variants were sterile filtered using a 0.22μm filter (Millipore). The concentration of the sterile samples were then quantified by A<sub>280</sub> and confirmed by BCA measurements (Pierce). For the variants digested with the protease in vitro, the digests were carried out as described in the digestion procedure for each protease. The digests were then diluted in the 1000 ng•ml-¹ dilution and sterile filtered. The Ricin and the undigested pAP214 in the pAP 214 cytotoxicity data were treated in the same manner but without the Cathepsin B treatment. Ricin and Ricin variants were serially diluted to the following concentrations: 1000 ng•ml-¹, 100 ng•ml-¹, 10 ng•ml-¹, 1 ng•ml-¹, 0.1 ng•ml-¹, 0.01 ng•ml-¹, 0.001 ng•ml-¹ with media containing 10%FBS and 1X pen/strep.

### Application of Toxin or Variants to Plates

Columns 2 to 9 were labeled: control, 1000 ng•ml-1, 100 ng•ml-1, 10 ng•ml-1, 1 ng•ml-1, 0.1 ng•ml-1, 0.01 ng•ml-1, 0.001 ng•ml-1 consecutively. The media was removed from all the sample wells with a multichannel pipettor. For each plate of variant and toxin, 50µl of media was added to wells 2B to 2G as the control, and 50µl of each sample dilution was added to the corresponding columns. For the pAP220 + MMP-9 data, the plates were incubated for one hour at 37(C with 5% CO<sub>2</sub>, then washed once and replaced with media, then incubated for 48 hours at 37(C with 5% CO<sub>2</sub>. For the pAP 214 + Cathepsin B data, the toxin was left on the plates and incubated for 24 hours at 37(C with 5% CO<sub>2</sub>, then 50 µl of media was added to the wells with the toxin and incubated for another 24 hours at 37(C with 5% CO<sub>2</sub>.

#### Sample Application

The whole amount of media (and/or toxin)was removed from each well with a multichannel pipettor, and replaced with 100  $\mu$ l of the substrate mixture (Promega Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit). The plates were incubated at 37(C with 5% CO<sub>2</sub> for 2 to 4 hours, and subsequently read with a Spectramax 340 96 well plate reader at 490nm. The IC<sub>50</sub> values were calculated using the GRAFIT software program.

#### **Results**

In experiments with pAP-214 and Cathepsin B incubated with COS-1 cells, it may be seen that cells incubated with pAP-214 alone, pAP-214 was ineffective at causing cell death (see Figure 56). However, the cytotoxicity of pAP-214 digested with Cathepsin B behaves similarly to the ricin control in COS-1 cells. This is also illustrated in Figure 56. Similarly, the cytotoxicity of undigested pAP-220 when incubated with COS-1 cells is lower than the cytotoxicity observed with COS-1 cells incubated with pAP-220 digested with MMP-9. Indeed the results suggest that the toxicity of digested pAP-220 is greater than that of ricin. (See Figure 57).

#### EXAMPLE 9

# 20 Procedure for Examining the Cytotoxicity of Ricin and Ricin Variants on Various Tissue Culture Cell Lines

#### **Cell Preparation**

After washing with 1XPBS (1.37M NaCl, 26.8mM KCl, 81mM Na<sub>2</sub>HPO<sub>4</sub>, 14.7mM KH<sub>2</sub>PO<sub>4</sub>), cells in log phase growth were removed from plates with 1X trypsin/EDTA (Gibco/BRL). The cells were centrifuged at 1100 rpm for 3 min, resuspended in media containing 10%FBS and 1X pen/strep (media used depended on the cell line being tested), and then counted using a haemocytometer. They were adjusted to a concentration of 5 X 10<sup>4</sup> cells•ml<sup>-1</sup> (faster growing cell lines were adjusted to 2 X10<sup>4</sup> cells•ml<sup>-1</sup>). One hundred microliters per well of cells was added to wells 2B - 2G through to wells 9B - 9G of a Falcon 96 well

tissue culture plate. A separate 96 well tissue culture plate was used for each sample of Ricin or Ricin variant. The plates were incubated at  $37(C \text{ with } 5\% \text{ CO}_2 \text{ for } 24 \text{ hours}.$ 

#### **Toxin Preparation**

The Ricin and Ricin variants were sterile filtered using a 0.22μm filter (Millipore). The concentration of the sterile samples were then quantified by A<sub>280</sub> and confirmed by a BCA measurement (Pierce). Ricin and Ricin variants were serially diluted to the following concentrations: 3000 ng•ml<sup>-1</sup>, 300 ng•ml<sup>-1</sup>, 30 ng•ml<sup>-1</sup>, 3 ng•ml<sup>-1</sup>, 0.3 ng•ml<sup>-1</sup>, 0.03ng•ml<sup>-1</sup>, 0.003 ng•ml<sup>-1</sup> with media containing 10%FBS and 1X pen/strep.

### Application of Toxin or Variants to Plates

Columns 2 to 9 were labeled: control, 0.001 ng•ml-1, 0.01 ng•ml-1, 0.1 ng•ml-1, 1ng•ml-1, 10 ng•ml-1, 100 ng•ml-1, 1000 ng•ml-1 consecutively. For each plate of variant and toxin, 50µl of media was added to wells 2B to 2G as the control, and 50µl of each sample dilution was added to the corresponding columns containing 100µl per well of cells (i.e. 50 µl of the 3000 ng•ml-1 dilution added to the wells B-G in column 9, labeled 1000 ng•ml-1). The plates were incubated for 48 hours at 37(C with 5% CO<sub>2</sub>.

## Sample Application

An amount of 140µl was removed from each well with a multichannel pipettor, and replaced with 100 µl of the substrate mixture (Promega Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation 25 Assay Kit). The plates were incubated at 37(C with 5% CO<sub>2</sub> for 2 to 4 hours, and subsequently read with a Spectramax 340 96 well plate reader at 490nm. The IC<sub>50</sub> values were calculated using the GRAFIT software program.

#### Results

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Referring to Table 2, it may be seen that the survival of cells is correlated with the proricin variant and the cell specific protease produced by the cell type. For example, in the HT1080 cell line, both pAP-214 and pAP-220 required only 2-1/2 times the amount of ricin to achieve the same level of cytotoxicity. On the other hand, pAP-224 required 193 times the amount of ricin to achieve the same level of cell death. As well, it may be seen that in the cells where expression of Cathepsin D is found, pAP-214 and 220 were more effective at causing cell death than ricin and more effective than pAP-224. Details concerning the various cells types used in these experiments are outlined below.

#### COS-1 (African Green Monkey Kidney Cells)

This is an SV40 transformed cell line which was prepared from established simian cells CV-1. (Reference: Gluzman, Y. (1975) Cell, 23, 175 - 182)(ATCC CRL 1650)

### HT-1080 Human Fibrosarcoma

(ATCC CCL 121) This cell line was shown to produce active MMP-9 in tissue culture. References: Moore et al. (1997) Gynecologic Oncology 65, 83-88.

#### 20 <u>9L Rat Glioblastoma</u>

Glioblastomas are generally associated with cathepsin B expression. Levels of cathepsin B expression correspond to the extent of progression of malignancy i.e. highest levels for glioblastomas over anaplastic astrocytomas over low-grade gliomas and normal brain tissue. The 9L cell line was provided by Dr. William Jia of the B.C. Cancer Agency.

References: Mikkelsen et al. (Aug. 1995) Journal of Neurosurgery 83(2), 285-290. Nakano et al. (1995) J. of Neurosurgery 83(2), 298-307.

## MCF-7 Human Breast Cancer Cell Line (Epithilial)

(ATCC CRL 1555) In the absence of estrogen cathepsin B has not been shown to be elevated relative to normal cells. It can be induced with estrogen to produce Cathepsin D. Production of MMP-9 is unknown.

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated to those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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## FULL CITATIONS FOR CERTAIN REFERENCES REFERRED TO IN THE SPECIFICATION

Bever Jr., C.T., Panitch, H.S., and Johnson, K.P. (1994) Neurology 44(4), 745-8. Increased cathepsin B activity in peripheral blood mononuclear cells of multiple sclerosis patients.

Cohen, P., Graves, H.C., Peehl, D.M., Kamarei, M., Giudice, L.C., and Rosenfeld, R.G. (1992) Journal of Clinal Endocrinology and Metabolism 75(4), 1046-53. Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma.

10 Conover, C.A. and De Leon, D.D. (1994) J. Biol. Chem. 269(10), 7076-80. Acid activated insulin-like growth factor-binding protein-3 proteolysis in normal and transformed cells. Role of cathepsin D.

Hansen, G., Schuster, A., Zubrod, C., and Wahn, V. (1995) Respiration 62(3), 117-24. Alpha 1-proteinase inhibitor abrogates proteolytic and secretagogue activity of cystic fibrosis sputum.

Muller, H.L., Oh, Y., Gargosky, S.E., Lehrnbecher, T., Hintz, R.L., and Rosenfeld, R.G. (1993) Journal of Clinical Endocrinology and Metabolism 77(5), 1113-9. Concentrations of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3), IGF, and IGFBP-3 protease activity in cerebrospinal fluid of children with leukemia, central nervous system tumor, or meningitis.

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TABLE 1

Tabl I - Sequence and Location of Oligonucieotide Primers

Name f Primer	Primer Sequence †	Corresponds t preproricin nucleotide numbers: (see
Ricin-109	5'- GGAGATGAAACCGGGAGGAAATACTATTGTAAT-3'	Figures 8-10)
Ricin-99Eco		27 to 59 37 to 59
Ricin267	5'- ACGGTTTATTTTAGTTGA-3'	300 to 317
Ricin486	5'- ACTTGCTGGTAATCTGAG -3'	519 to 536
Ricin725	5'- AGAATAGTTGGGGGAGAC -3'	758 to 775
Ricin937	5'- AATGCTGATGTTTGTATG -3'	970 to 987
Ricin 1151	5'- CGGGAGTCTATGTGATGA -3'	1184 to 1201
Ricin1399	5'-GCAAATAGTGGACAAGTA -3'	1432 to 1449
Ricin 1627	5'- GGATTGGTGTTAGATGTG -3'	1660 to 1677
Ricin 1729C	5'- ATAACTTGCTGTCCTTTCA -3'	1864 to 1846
Ricin1729C Xba	5'- CGCTCTAGATAACTTGCTGTCCTTTCA	1864 to 1846

Tunderlined sequences inserted for subcloning purposes and not included in final preproricin sequences

Table 2: <u>Comparative Toxicities to Selected Cell Lines of Ricin and Ricin Provariants</u>

Cell Line	IC50 <sub>Ricin</sub> (ng/ml)	IC50 <sub>pAP214</sub> IC50 <sub>Ricin</sub>	IC50 <sub>pAP220</sub> IC50 <sub>Ricin</sub>	IC50 <sub>pAP224</sub> IC50 <sub>Ricin</sub>
COS-1	0.1	17	22	150
HT1080	0.5	2.46	2.14	193
9L	10.8	1.3	1.7	32.3
MCF-7 (without estrogen)	0.09	27.8	40	742

#### I CLAIM:

- 1. A purified and isolated nucleic acid having a nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence linking the A and B chains, the heterologous linker sequence containing a cleavage recognition site for a disease-specific protease.
- 2. The nucleic acid sequence of claim 1 wherein the linker sequence contains a cleavage recognition site recognized by a protease selected from the group consisting of: a cancer associated protease, a viral protease, a fungal protease, and a parasite protease.
- 3. A nucleic acid sequence of claim 2 wherein the A chain is ricin A chain, abrin toxin A chain, diphtheria toxin A chain, or Domain I of Pseudomonas endotoxin.
- A nucleic acid sequence of claim 2 wherein the A chain is
   volkensin toxin A chain, cholera toxin A chain, modeccin toxin A chain or shiga toxin A chain.
  - 5. A nucleic acid sequence of claim 2 wherein the B chain is ricin B chain, abrin toxin A chain, diphtheria toxin B chain, or Domain II of Pseudomonas endotoxin.
- 20 6. A nucleic acid sequence of claim 2 wherein the B chain is volkensin toxin B chain, cholera toxin B chain, modeccin toxin B chain or shiga toxin B chain.
- A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by a cancer-associated protease which is
   selected from the group consisting of: cathepsin B, an Epstein-Barr

virus-specific protease, a matrix metalloproteinase, cathespin L, cathespin D, urokinase-type plasminogen activator, tissue-type plasminogen activator, human prostate-specific antigen, kallikrein, neutrophil elastase, and calpain.

- 5 8. A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by a parasitic protease which is a Plasmodium falciparum protease.
- 9. A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by viral protease which is selected from the group consisting of: human cytomegalovirus, human herpes virus, varicella zoster virus, hepatitis A virus, hepatitis C virus, and infectious laryngotracheitis virus.
- 10. A nucleic acid sequence of claim 2 wherein the cleavage recognition site is recognized by fungal protease which is a *Candida* acid15 protease.
- 11. A nucleic acid sequence of claim 2 having the nucleotide sequence according to SEQ ID No. 3; SEQ ID No 5; SEQ ID No 7; SEQ ID No 9; SEQ ID No 11; SEQ ID No 13; SEQ ID No 15; SEQ ID No 17; SEQ ID No 19; SEQ ID No 21; SEQ ID No 23; SEQ ID No 25; SEQ ID No 27; SEQ ID No 29; SEQ ID No 31; SEQ ID No 33; SEQ ID No 35; SEQ ID No 37; SEQ ID No 39; SEQ ID No 48; SEQ ID No 50; SEQ ID No 52; SEQ ID No 54; SEQ ID No 74; SEQ ID No 77; SEQ ID No 80; SEQ ID No 83; SEQ ID No 86; SEQ ID No 89; SEQ ID No 92; SEQ ID No 95; SEQ ID No 98; SEQ ID No 101; SEQ ID No 104; SEQ ID No 107; SEQ ID No 110; SEQ ID No 122; or SEQ ID No 125.
  - 12. A plasmid incorporating the nucleic acid of claim 1 to 11.

- 13. A baculovirus transfer vector incorporating the nucleic acid of claim 1 to 11.
- 14. A recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for a disease-specific protease.
- 15. The recombinant protein of claim 14 wherein the linker sequence contains a cleavage recognition site which is recognized by a protease selected from the group consisting of: a cancer, viral, fungal, and a parasitic protease.
- 16. A recombinant protein of claim 14 wherein the A chain is ricin A chain, abrin toxin B chain, diphtheria toxin A chain, or Domain I of Pseudomonas endotoxin.
- 17. A recombinant protein of claim 14 wherein the A chain is15 volkensin toxin A chain, cholera toxin A chain, modeccin toxin A chain or shiga toxin A chain.
  - 18. A recombinant protein of claim 14 wherein the B chain is ricin B chain, abrin toxin B chain, diphtheria toxin B chain, or Domain II of Pseudomonas endotoxin.
- 20 19. A recombinant protein of claim 14 wherein the B chain is volkensin toxin B chain, cholera toxin B chain, modeccin toxin B chain or shiga toxin B chain.
  - 20. A recombinant protein of claim 14 wherein the cleavage recognition site is recognized by a cancer-associated protease selected

from the group consisting of: cathepsin B, an Epstein-Barr virus-specific protease, a matrix metalloproteinase, cathespin L, cathespin D, urokinase-type plasminogen activator, tissue-type plasminogen activator, human prostate-specific antigen, kallikrein, neutrophil elastase, and calpain.

- 21. A recombinant protein of claim 14 wherein the cleavage recognition site is recognized by a parasitic protease which is a Plasmodium falciparum protease.
- 22. A recombinant protein of claim 14 wherein the cleavage recognition site is recognized by a viral protease which is selected from the group consisting of: human cytomegalovirus, human herpes virus, varicella zoster virus, hepatitis A virus, hepatitis C virus and infectious laryngotracheitis virus.
- 23. A recombinant protein of claim 14 wherein the cleavage recognition site is recognized by a fungal protease which is a Candida acid protease.
- 24. A recombinant protein of claim 14 having the linker amino acid sequence according to SEQ ID No. 40; SEQ ID No. 41; SEQ ID No. 42; SEQ ID No. 43; SEQ ID No. 44; SEQ ID No. 45; SEQ ID No. 46; SEQ ID No. 55;
  20 SEQ ID No. 56; SEQ ID No. 57; SEQ ID No. 58; SEQ ID No. 59; SEQ ID No. 60; SEQ ID No. 61; SEQ ID No. 62; SEQ ID No. 63; SEQ ID No. 64; SEQ ID No. 65; SEQ ID No. 66; SEQ ID No. 67; SEQ ID No. 68; SEQ ID No. 69; SEQ ID No. 70; SEQ ID No. 71; SEQ ID No. 72; SEQ ID No. 75; SEQ ID No. 78; SEQ ID No. 81; SEQ ID No. 84; SEQ ID No. 87; SEQ ID No. 90; SEQ ID No. 93; SEQ ID No. 96; SEQ ID No. 99; SEQ ID No. 102; SEQ ID No. 105; SEQ ID No. 108; SEQ ID No. 111; SEQ ID No. 114; SEQ ID No. 117; SEQ ID No. 120; SEQ ID No. 123; or SEQ ID No. 126.

- 25. A method of inhibiting or destroying cells affected by a disease, which cells are associated with a protease specific to the disease comprising the steps of:
- (a) preparing a purified and isolated nucleic acid having a nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin, and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for the protease;
- (b) introducing the nucleic acid into a host cell and expressing the nucleic acid in the host cell to obtain a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a linker amino acid sequence;
  - (c) suspending the protein in a pharmaceutically acceptable carrier, diluent or excipient, and
- (d) contacting the cells with the recombinant protein.
  - 26. The method of claim 25 where the disease is one of cancer or cells infected with a fungus, virus or parasite.
- 27. A method of inhibiting or destroying cells affected by a disease, which cells are associated with a protease specific to the disease
  20 comprising the step of contacting the cells with a recombinant protein according to anu one of claims 14 to 24.
  - 28. A method of treating a disease comprising administering a recombinant protein according to any one of claims 14 to 24 to an animal in need thereof.
- 25 29. A method of treating a disease comprising administering a nucleic acid molecule according to any one of claims 2 to 11 to an animal in need thereof.

- 30. A method of treating a mammal with cancer or infected with a fungus, virus or parasite, comprising the steps of preparing a recombinant protein of claim 14 wherein the linker sequence contains a cleavage recognition site for a cancer, fungal, viral or parasitic protease and administering the protein to the mammal.
- 31. A process for preparing a pharmaceutical for treating a mammal with cancer, fungal infection, viral infection or parasitic infection, comprising the steps of :
- (a) preparing a purified and isolated nucleic acid having a 10 nucleotide sequence encoding an A chain of a ricin-like toxin, a B chain of a ricin-like toxin, and a heterologous linker amino acid sequence, linking the A and B chains, wherein the linker sequence contains a cleavage recognition site for a cancer, viral or parasitic protease;
- (b) introducing the nucleic acid into a host cell and expressing 15 the nucleic acid in the host cell to obtain a recombinant protein comprising an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a linker amino acid sequence;
  - (c) suspending the protein in a pharmaceutically acceptable carrier, diluent or excipient.
- 20 32. A use of a recombinant protein according to any one of claims 14 to 24 to treat a disease.
  - A use of a nucleic acid molecule according to any one of claims 1 to 11 to treat a disease.
- 34. A pharmaceutical composition for treating cancer or a fungal, or viral, or parasitic infection in an animal comprising the recombinant protein of claim 14 and a pharmaceutically acceptable carrier, diluent or excipient.

35. A pharmaceutical composition for treating cancer or a fungal, or viral, or parasitic infection in an animal comprising the nucleic acid molecule of claim 2 and a pharmaceutically acceptable carrier, diluent or excipient.

## FIGURE 1

## Complete Sequence of Baculovirus Transfer Vector, pVL1393

```
preliminary; circular DNA; SYN;
ID
     PVL1393
9632 BP.
XX
AC
     IG1137;
XX
     01-FEB-1993 (Rel. 7, Created)
DT
     01-JUL-1995 (Rel. 12, Last updated, Version
DT
1)
XX
     E. coli plasmid vector pVL1393 - complete.
DE
XX
KW
     cloning vector.
XX
os
     Cloning vector
     Artificial sequences; Cloning vehicles.
OC
XX
RN
     [1]
RC
     p2Bac from baculovirus
RC
     p2Blue from p2Bac
RC
     pBlueBac from AcNPV
RC
     pBlueBac2 from AcNPV
RC
     pBlueBacIII from AcNPV
RC
     pBlueBacHisA from AcNPV
RC
     pBlueBacHisB from AcNPV
RC
     pBlueBacHisC from AcNPV
RC
     pVL1392, pVL1393 from pAc360
RA
RT
RL
     The Digest 5:2-2(1992).
XX
CC
     NM (pVL1393)
CC
     CM (yes)
CC
     NA (ds-DNA)
CC
     TP (circular)
CC
     ST ()
CC
     TY (plasmid)
CC
      SP (British
Biotechnology) (Invitrogen)
     HO (E.coli NM522) (E.coli
INValphaF')(insect)
CC
      CP ()
CC
      FN (expression) (transfer)
CC
      SE ()
CC
      PA (pAC360)
CC
      BR (pVL1392)
CC
      OF ()
CC
      OR ()
XX
FH
      Key
                       Location/Qualifiers
 FH
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FT

## 2/254

## FIGURE 1 (Cont'd)

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polyhedrin gene
FT
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     transposon
FT
                      0..0
FT
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FT
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     misc_binding
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FT
     misc_binding
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FT
                      /note="SIT XhoI"
FT
     promoter
                      0..0
FT
                      /note="PRO AcMNPV polyhedrin gene"
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FT
                      0..0
FT
                      /note= *MCS
FT
                      BamHI-SmaI-XbaI-EcoRI-NotI-XmaIII-PstI-
BglII*
FT
     rep_origin
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FT
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pBR322) *
FT
     CDS
                      complement(0..0)
FT
                      /note="ANT E. coli beta-lactamase gene
(bla)
FT
                      ampicillin resistance gene (apr/amp)*
XX
     Sequence 9632 BP; 2602 A; 2122 C; 2176 G; 2732 T; 0
SO
other;
     aagctttact cgtaaagcga gttgaaggat catatttagt tgcgtttatg
     agataagatt gaaagcacgt gtaaaatgtt tcccgcgcgt tggcacaact
     atttacaatg cggccaagtt ataaaagatt ctaatctgat atgttttaaa
     acacctttgc ggcccgagtt gtttgcgtac gtgactagcg aagaagatgt
     gtggaccgca gaacagatag taaaacaaaa ccctagtatt ggagcaataa
     togatttaac caacacgtot aaatattatg atggtgtgca ttttttgcgg
     gegggeetgt tatacaaaaa aatteaagta eetggeeaga etttgeegee
     tgaaagcata gttcaagaat ttattgacac ggtaaaagaa tttacagaaa
     agtgtcccgg catgttggtg ggcgtgcact gcacacacgg tattaatcgc
     accepttaca tegtetecae atattaate cacacceteg gtattecec
     gcaggaagcc atagatagat tcgaaaaagc cagaggtcac aaaattgaaa
     gacaaaatta cgttcaagat ttattaattt aattaatatt atttgcattc
     tttaacaaat actttateet atttteaaat tgttgegett etteeagega
     accaaaacta tgcttcgctt gctccgttta gcttgtagcc gatcagtggc
     gttgttccaa tcgacggtag gattaggccg gatattctcc accacaatgt
     tggcaacgtt gatgttacgt ttatgctttt ggttttccac gtacgtcttt
     tggccggtaa tagccgtaaa cgtagtgccg tcgcgcgtca cgcacaacac
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     atttcgtctt tcttttgcat ggtttcctgg aagccggtgt acatgcggtt
     tagatcagtc atgacgcgcg tgacctgcaa atctttggcc tcgatctgct
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    tecteggitt titgegeeae cacegettge agegegittg tgtgeteggt
     gaatgtcgca atcagcttag tcaccaactg tttgctctcc tcctcccgtt
     gtttgatcgc gggatcgtac ttgccggtgc agagcacttg aggaattact
     tettetaaaa gecattettg taattetatg gegtaaggea atttggaett
```

## FIGURE 1 (Cont'd)

cataatcagc tgaatcacgc cggatttagt aatgagcact gtatgcggct gcaaatacag cgggtcgccc cttttcacga cgctgttaga ggtagggccc ccattttgga tggtctgctc aaataacgat ttgtatttat tgtctacatg aacacgtata gctttatcac aaactgtata ttttaaactg ttagcgacgt ccttggccac gaaccggacc tgttggtcgc gctctagcac gtaccgcagg ttgaacgtat cttctccaaa tttaaattct ccaattttaa cgcgagccat tttgatacac gtgtgtcgat tttgcaacaa ctattgtttt ttaacgcaaa ctaaacttat tgtggtaagc aataattaaa tatgggggaa catgcgccgc tacaacactc gtcgttatga acgcagacgg cgccggtctc ggcgcaagcg gctaaaacgt gttgcgcgtt caacgcggca aacatcgcaa aagccaatag tacagttttg atttgcatat taacggcgat tttttaaatt atcttattta ataaatagti atgacgeeta caacteeeeg ceegegttga etegetgeae ctcgagcagt tcgttgacgc cttcctccgt gtggccgaac acgtcgagcg ggtggtcgat gaccagcggc gtgccgcacg cgacgcacaa gtatctgtac accgaatgat cgtcgggcga aggcacgtcg gcctccaagt ggcaatattg gcaaattcga aaatatatac agttgggttg tttgcgcata tctatcgtgg cgttgggcat gtacgtccga acgttgattt gcatgcaagc cgaaattaaa tcattgcgat tagtgcgatt aaaacgttgt acatcctcgc ttttaatcat gccgtcgatt aaatcgcgca atcgagtcaa gtgatcaaag tgtggaataa tgttttcttt gtattcccga gtcaagcgca gcgcgtattt taacaaacta gccatcttgt aagttagttt catttaatgc aactttatcc aataatatat tatgtatege aegteaagaa ttaacaatge gecegttgte geateteaae acgactatga tagagatcaa ataaagcgcg aattaaatag cttgcgacgc aacgtgcacg atctgtgcac gcgttccggc acgagetttg attgtaataa gtttttacga agcgatgaca tgacccccgt agtgacaacg atcacgccca aaagaactgc cgactacaaa attaccgagt atgtcggtga cgttaaaact attaagccat ccaatcgacc gttagtcgaa tcaggaccgc tggtgcgaga agccgcgaag tatggcgaat gcatcgtata acgtgtggag tccgctcatt agagegteat gtttagacaa gaaagetaca tatttaattg atcccgatga ttttattgat aaattgaccc taactccata cacggtattc tacaatggcg gggttttggt caaaatttcc ggactgcgat tgtacatgct gttaacggct cogoccacta ttaatgaaat taaaaattoo aattttaaaa aacgoagoaa gagaaacatt tgtatgaaag aatgcgtaga aggaaagaaa aatgtcgtcg acatgetgaa caacaagatt aatatgeete egtgtataaa aaaaatattg aacgattīga aagaaaacaa tgtaccgcgc ggcggtatgt acaggaagag gtttatacta aactgttaca ttgcaaacgt ggtttcgtgt gccaagtgtg aaaaccgatg tttaatcaag gctctgacgc atttctacaa ccacgactcc aagtgtgtgg gtgaagtcat gcatctttta atcaaatccc aagatgtgta taaaccacca aactgccaaa aaatgaaaac tgtcgacaag ctctgtccgt ttgctggcaa ctgcaagggt ctcaatccta tttgtaatta ttgaataata gcaacaagaa cattigtagt attatctata attgaaaacg cgtagttata atcgctgagg taatatttaa aatcattttc aaatgattca cagttaattt gcgacaatat aattttattt tcacataaac tagacgcctt gtcgtcttct tettegtatt cettetett tteatttte teeteataaa aattaacata gttattatcg tatccatata tgtatctatc gtatagagta aatttttgt tgtcataaat atatatgtct tttttaatgg ggtgtatagt accgctgcgc atagtttttc tgtaatttac aacagtgcta ttttctggta gttcttcgga gtgtgttgct ttaattatta aatttatata atcaatgaat ttgggatcgt cggttttgta caatatgttg ccggcatagt acgcagette ttetagttea attacaccat tttttageag caceggatta acataacttt ccaaaatgtt gtacgaaccg ttaaacaaaa acagttcacc tecetttet atactattgt etgegageag ttgtttgttg ttaaaaataa cagecattgt aatgagaege acaaactaat atcacaaact ggaaatgtet

## FIGURE 1 (Cont'd)

ctgtcccgat ttatttgaaa cactacaaat taaaggcgag ctttcgtacc aacttgttag caatattatt agacagctgt gtgaagcgct caacgatttg cacaagcaca atttcataca caacgacata aaactcgaaa atgtcttata tttcgaagca cttgatcgcg tgtatgtttg cgattacgga ttgtgcaaac acgaaaactc acttagcgtg cacgacggca cgttggagta ttttagtccg gaaaaaattc gacacacac tatgcacgtt tcgtttgact ggtacgcggc gtgttaacat acaagttgct aacgtaatca tggtcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga gccggaagca taaagtgtaa agcctggggt gcctaatgag tgagctaact cacattaatt gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga atcggccaac gcgcggggag aggcggtttg cgtattgggc getetteege tteetegete actgactege tgegeteggt egtteggetg cggcgagcgg tatcagctca ctcaaaggcg gtaatacggt tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc cccctgacg agcatcacaa aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc ctgccgctta ccggatacct gtccgccttt cteeettegg gaagegtgge gettteteat ageteaeget gtaggtatet cagttcggtg taggtcgttc gctccaagct gggctgtgtg cacgaaccc cegtteagee egacegetge geettateeg gtaactateg tettgagtee aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc tacagagttc ttgaagtggt ggcctaacta cggctacact agaaggacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt taagggattt tggtcatgag attatcaaaa aggatettea ectagateet tttaaattaa aaatgaagtt ttaaateaat ctaaagtata tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata cgggagggct taccatctgg ccccagtgct gcaatgatac cgcgagaccc acgctcaccg gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgcag aagtggtcct gcaactttat ccgcctccat ccagtctatt aattgttgcc gggaagctag agtaagtagt tegecagtta atagtttgeg caaegttgtt gecattgeta caggeategt ggtgteaege tegtegtttg gtatggette atteagetee ggttcccaac gatcaaggcg agttacatga tcccccatgt tgtgcaaaaa ageggttage teetteggte etcegategt tgteagaagt aagttggeeg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca aggatcttac cgctgttgag atccagttcg atgtaaccca ctcgtgcacc caactgatct tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt cctttttcaa tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt tagaaaaata aacaaatagg ggttccgcgc acattteece gaaaagtgee acetgaegte taagaaacea ttattateat gacattaacc tataaaaata ggcgtatcac gaggcccttt cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg gtcacagett gtctgtaage ggatgeeggg ageagacaag eeegteaggg

## FIGURE 1 (Cont'd)

atcaatatat agttgctgat atcatggaga taattaaaat gataaccatc tcgcaaataa ataagtattt tactgttttc gtaacagttt tgtaataaaa aaacctataa atattccgga ttattcatac cgtcccacca tcgggcgcgg atcccgggta ccttctagaa ttccggagcg gccgctgcag atctgatcct ttcctgggac ccggcaagaa ccaaaaactc actctcttca aggaaatccg taatgttaaa cccgacacga tgaagcttgt cgttggatgg aaaggaaaag agttetacag ggaaacttgg accegettea tggaagacag ettecceatt gttaacgacc aagaagtgat ggatgttttc cttgttgtca acatgcgtcc cactagacce aaccettett acaaatteet gecceaacac getetgeett gcgaccccga ctatgtacct catgacgtga ttaggatcgt cgagccttca tgggtgggca gcaacaacga gtaccgcatc agcctggcta agaagggcgg cggctgccca ataatgaacc ttcactctga gtacaccaac tcgttcgaac agttcatcga tcgtgtcatc tgggagaact tctacaagcc catcgtttac ateggtaceg actetgetga agaggaggaa atteteettg aagttteeet ggtgttcaaa gtaaaggagt ttgcaccaga cgcacctctg ttcactggtc cggcgtatta aaacacgata cattgttatt agtacattta ttaagcgcta gattetgtge gttgttgatt tacagacaat tgttgtacgt attttaataa ttcattaaat ttataatctt tagggtggta tgttagagcg aaaatcaaat gattttcagc gtctttatat ctgaatttaa atattaaatc ctcaatagat ttgtaaaata ggtttcgatt agtttcaaac aagggttgtt tttccgaacc gatggctgga ctatctaatg gattttcgct caacgccaca aaacttgcca tgtaataaag gttcgacgtc gttcaaaata ttatgcgctt ttgtatttct ttcatcactg tcgttagtgt acaattgact cgacgtaaac acgttaaata aagcttggac atatttaaca tcgggcgtgt tagctttatt aggccgatta tegtegtegt eccaaceete gtegttagaa gttgetteeg aagacgattt tgccatagcc acacgacgcc tattaattgt gtcggctaac acgtccgcga tcaaatttgt agttgagctt tttggaatta tttctgattg cgggcgtttt tgggcgggtt tcaatctaac tgtgcccgat tttaattcag acaacacgtt agaaagcgat ggtgcaggcg gtggtaacat ttcagacggc aaatctacta atggcggcgg tggtggagct gatgataaat ctaccatcgg tggaggcgca ggcggggctg gcggcggagg cggaggcgga ggtggtggcg gtgatgcaga cggcggttta ggctcaaatg tctctttagg caacacagtc ggcacctcaa ctattgtact ggtttcgggc gccgtttttg gtttgaccgg tctgagacga gtgcgatttt tttcgtttct aatagcttcc aacaattgtt gtctgtcgtc taaaggtgca gcgggttgag gttccgtcgg cattggtgga gcgggcggca attcagacat cgatggtggt ggtggtggtg gaggcgctgg aatgttaggc acgggagaag gtggtggcgg cggtgccgcc ggtataattt gttctggttt agtttgttcg cgcacgattg tgggcaccgg cgcaggcgcc gctggctgca caacggaagg tcgtctgctt cgaggcagcg cttggggtgg tggcaattca atattataat tggaatacaa atcgtaaaaa tctgctataa gcattgtaat ttcgctatcg tttaccgtgc cgatatttaa caaccgctca atgtaagcaa ttgtattgta aagagattgt ctcaagctcg ccgcacgccg ataacaagcc ttttcatttt tactacagca ttgtagtggc gagacacttc gctgtcgtcg acgtacatgt atgctttgtt gtcaaaaacg tcgttggcaa gctttaaaat atttaaaaga acatctctgt tcagcaccac tgtgttgtcg taaatgttgt ttttgataat ttgcgcttcc gcagtatcga cacgttcaaa aaattgatgc gcatcaattt tgttgttcct attattgaat aaataagatt gtacagattc atatctacga ttcgtcatgg ccaccacaaa tgctacgctg caaacgctgg tacaatttta cgaaaactgc aaaaacgtca aaactcggta taaaataatc aacgggcgct ttggcaaaat atctatttta tcgcacaagc ccactagcaa attgtatttg cagaaaacaa tttcggcgca caattttaac gctgacgaaa taaaagttca ccagttaatg agcgaccacc caaattttat aaaaatctat tttaatcacg gttccatcaa caaccaagtg atcgtgatgg actacattga

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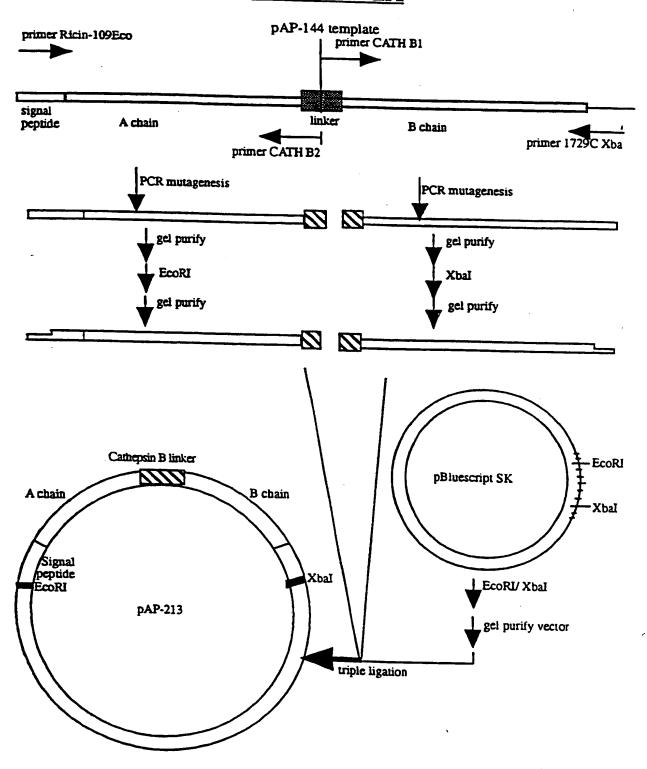
## FIGURE 1 (Conf'd)

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**bCT/CV98/00394** 

11E64/86 OM

## FIGURE 2A



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WT preproricin linker

primer CATH-B1

5'- ATGGTGCCAAATTTTAAT.3

- TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT. -- AGAAACGAATATTÇÇĞT|CACCACGGTTTAAAATTA

primer CATH-B2

3 · - TCTCGATTTAAGCAAAGAAAACTG-5

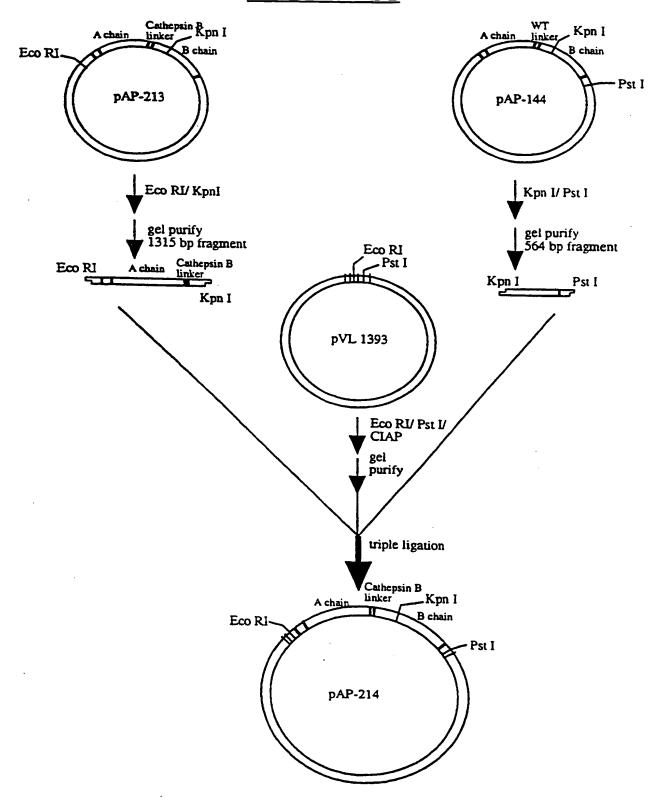
PCR mutagenesis

ligate with pBluescript SK

pAP 213 linker (Cathepsin-B variant) TCTTTGCTTAAATCGAGAATGGTGCCAAATTTTAAT—AGAAACGAATTTAGCTCTTACCACGGTTTAAAATTA—

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## FIGURE 2C



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## FIGURE 2D

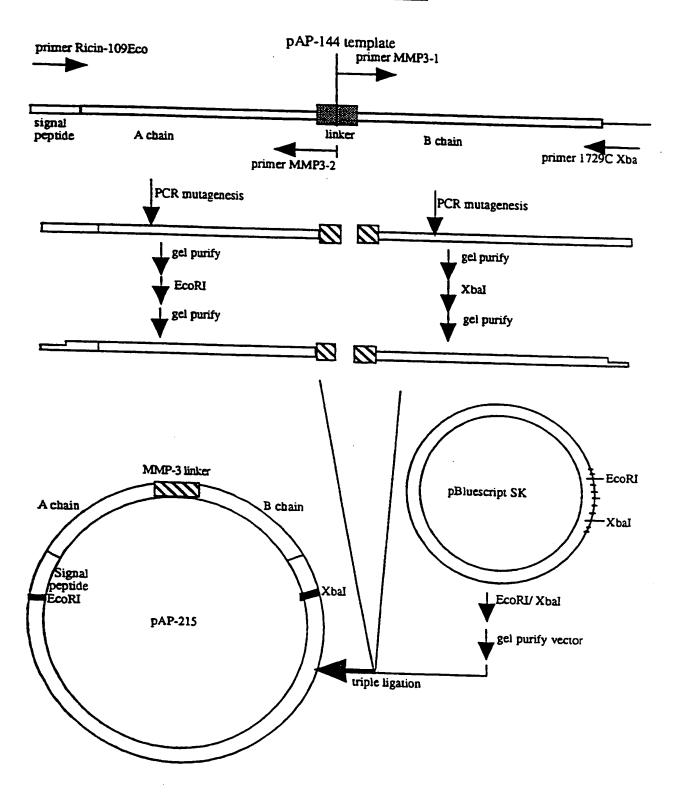
	10	20	30	40	5 ọ
1	GAATTCATGAAA CTTAAGTACTTT	CCGGGAGGAAAT GGCCCTCCTTTA	ACTATTGTAA TGATAACATT	TATGGATGTA TATACTACAT	 TGCAGT ACGTCA
51	GGCAACATGGCT	TTGTTTTGGATC	CACCTCAGGG	TGGTCTTTCA	CATTAG
	CCGTTGTACCGA	DATCOAAAAACAA	GTGGAGTCC	ACCAGAAAGT	GTAATC
101	AGGATAACAACA	TATTCCCCAAAC	CAATACCCAAT	TTATAAACTTT	ACCACA
	TCCTATTGTTGT	ATAAGGGGTTTC	TTATGGGTTF	AAAƏTTTAAA	TGGTGT
151	GCGGGTGCCACT	GTGCAAAGCTAC	CACAAACTTTI	ATCAGAGCTGT	TCGCGG
	CGCCCACGGTGA	CACGTTTCGATG	STGTTTGAAA	FAGTCTCGACA	AGCGCC
201	TCGTTTAACAAC	TGGAGCTGATGT	rgagacatga?	TATACCAGTGT	TGCCAA
	AGCAAATTGTTG	ACCTCGACTAC	Actetgtacti	ATATGGTCACA	ACGGTT
251	ACAGAGTTGGTT	TGCCTATAAAC(	CAACGGTTTA!	TTTAGTTGAA	CTCTCA
	TGTCTCAACCAA	ACGGATATTTG(	STTGCCAAAT!	TTOAACTT	GAGAGT
301	AATCATGCAGAG	CTTTCTGTTACI	ATTAGCGCTG(	GATGTCACCAA	TGCATA
	TTAGTACGTCTC	GAAAGACAATG	FAATCGCGAC(	CTACAGTGGTT	ACGTAT
351	TGTGGTCGGCTA	CCGTGCTGGAA	ATAGCGCATA'	TTTCTTTCATC	CTGACA
	ACACCAGCCGAT	CGCACGACCTT	PATCGCGTAT	AAAGAAAGTAG	GACTGT
401	ATCAGGAAGATO	CAGAAGCAATC	ACTCATCTTT	TCACTGATGTT	CAAAAT
	TAGTCCTTCTAO	CTCTTCGTTAG	IGAGTAGAAA	AGTGACTACA2	GTTTTA
451	CGATATACATTO	GCCTTTGGTGG	TAATTATGAT.	AGACTTGAACA	ACTTGC
	GCTATATGTAA	GCGGAAACCACC	ATTAATACTA	TCTGAACTTGT	TTGAACG
501	TGGTAATCTGAC	AGAAAATATCG	AGTTGGGAAA	TGGTCCACTAC	AGGAGG
	ACCATTAGACTC	TCTTTTATAGC	TCAACCCTTT	ACCAGGTGATC	TCCTCC
551	CTATCTCAGCGG	TTTATTATTAC	AGTACTGGTG	GCACTCAGCT:	CCAACT
	GATAGAGTCGCG	SAAATAATAATG	TCATGACCAC	CGTGAGTCGA	AGGTTGA
601	CTGGCTCGTTC:	CTTTATAATTTG	CATCCAAATG	ATTTCAGAAG	CAGCAAG
	GACCGAGCAAG	GAAATATTAAAC	GTAGGTTTAC	TAAAGTCTTC	STCGTTC
651	ATTCCAATATA	TTGAGGGAGAAA	TGCGCACGAG	CATTAGGTACE	AACCGGA
	TAAGGTTATAT	AACTCCCTCTTT	ACGCGTGCTC	TAATTAGTT	ITGGCCT
701	GATCTGCACCA CTAGACGTGGT	GATCCTAGCGTA CTAGGATCGCAT	ATTACACTTG TAATGTGAAC	OTTOATAAOAS	GGGGAGA CCCCTCT
751	CTTTCCACTGC	AATTCAAGAGTC	TAACCAAGG	AGCCTTTGCTA	GTCCAAT
	GAAAGGTGACG	TTAAGTTCTCAG	TOOTTOOTTA	CGGGAAACGAT	CAGGTTA
801	TCAACTGCAAA	GACGTAATGGTT	CCAAATTCAC	TGTGTACGAT	GTGAGTA
	AGTTGACGTTT	CTGCATTACCAA	AGGTTTAAGTC	ACACATGCTA	CACTCAT
853	TATTAATCCCT	ATCATAGCTCTC	CATGGTGTATI	AGATGCGCACC	TCCACCA
	ATAATTAGGGA	TAGTATCGAGAC	STACCACATA:	PCTACGCGTGG	AGGTGGT
90:	1 TCGTCACAGTT	ATTOTTTOTTAT	ATCGAGAAT(	GGTGCCAAATT	TTAATGC
	AGCAGTGTCAA	AAGAAAAAAA	TTAGCTCTTA(	CCACGGTTTAA	AATTACG

# FIGURE 2D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	ACTACAAACATACCTAGGACTCGCCTATCACGTATCGTAGGTCGAAATG
	ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CACAMAGIGITAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
	The state of the s
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
	TO THE STATE OF TH
1101	CA A A CA
	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
	- TONCHARI IGAIGAATGO
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
	CCATGTCAGGCCTTAGATGTGTGTGTGTGTGCAATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTATGATACGTTATGACGACGT
1201	A CERTA MODEL COLOR
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCCTATCATCATCATCATCATCATCA
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTAGATCACATCACACACACACACACACACACACACAC
	GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1201	
1201	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
	THE CONTROL OF THE CON
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTA THE TEACHER THE TEACHER TO THE TEACHER THE THE TEACHER THE TEACHER THE TEACHER THE TEACHER THE TEACHER THE THE THE THE THE THE TEACHER THE
	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTCTTCATTCATTCATTCAGGACTGTAGCAGTGAAA
	THE TOTAL PROPERTY OF THE PROP
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TOOLIGARCAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCACAC
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
	TO THE CAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	THE TAXABLE TO A CONTROL OF TAXABLE TA
1551	TOTAL SOLMONDO
	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGAACACCGGGACGTAGGACGATGCTACAAACCAACGATGGAACACCAACAACAACCAAC
	THE TAGGAGACCEGITECTACCTACA
1601	TCAAGAATGATGCA ACCATTOTTA A ATTOTTA A A ATTOTTA A ATTOTTA A ATTOTTA A ATTOTTA A ATTOTTA A A A
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
	THE TACE TIGGTAAAATTTAAACATATCACCTAACCACAATCACAATCACAATCACAATCACAATCACAATCACAAACCACAATCACAATCACAAACCACAATCACAAACCACAAACCACAATCACAAACCACAAACCACAAACCACAAACCACAAACCACAAACCACA
1651	CECACOCA
T021	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
	CACTCCGCTAGCCTAGGCTCGGAATTTCTTTTTTTTTTT
	CACTCCGCTAGCCTAGGCTCGGAATTGTTTAGTATAGTA
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
	ACCA CONCENTRACE ARATA I GGTTACCATTATTTTGATAGACAGA TTACT
	ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA
9 T -	The state of the s
1751	CTCTTGCAGTGTGTGTCTCCCATCA A A A TO TO TO
	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	THE TOTAL PROPERTY OF THE PROP
1801	GCACATTOCTA A STORMAN
	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTCTCTCTCTCTC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
	TOTAL TOTAL CONTINUATION TO THE PROPERTY OF THE
1851	TGCAG
	ACGTC

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## FIGURE 3A



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FIGURE 3B

WT preproricin linker

primer MMP3-1

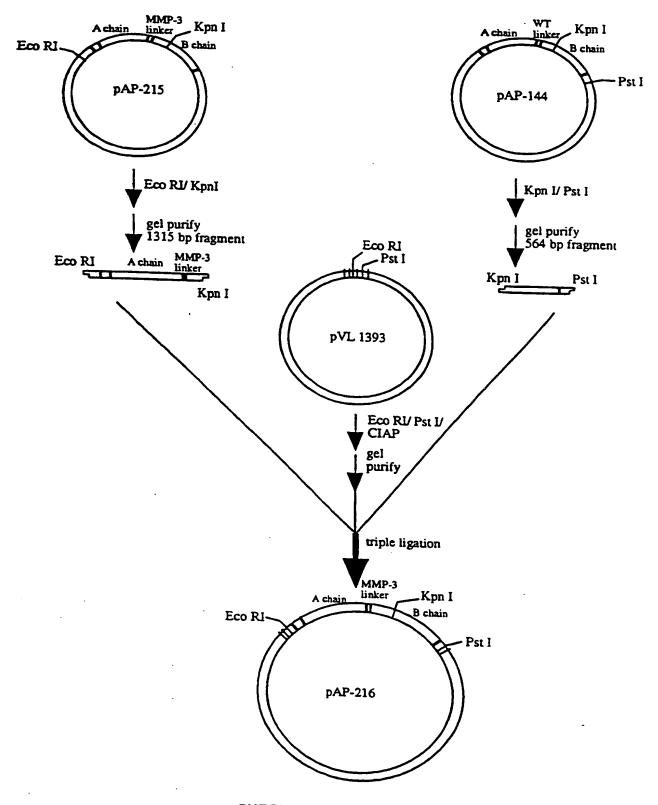
5'- TTTTTGGACTTATGAATGCTGATGTTTGT -3' TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT-GGTAGCAGTGTCAAAGCAGGCTTCGGTGTCGTT-5'

primer MMP3-2

PCR mutagenesis
Igate with pBluescript SK

pAP 215 linker (MMP-3 variant) 

## FIGURE 3C



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#### FIGURE 3D

30

	CTTAAGTACTTTGGCCCTCCTTTATGATAACATTATACCTACATACGTCA
51	GGCAACATCCCTTTTCTTTTTTTCCATCCATCCATCCATC
	CCGTTGTACCGAAACAAACCTAGGTGGAGTCCCACCAGAAAGTGTAATC
101	AGGATAACAACATATTTCCCCAAACAATA
	AGGATAACAATATTCCCCAAACAATACCCAATTATAAACTTTACCACA
	TCCTATTGTTGTATAAGGGGTTTGTTATGGGTTAATATTTGAAATGGTGT
151	GCGCGCCCA CTCTCCCA A A CCTTA CA
	GCGGGTGCCACTGTGCAAAGCTACACAAACTTTATCAGAGCTGTTCGCGG
	CGCCCACGGTGACACGTTTCGATGTGTTTGAAATAGTCTCGACAAGCGCC
201	TCGTTTAACA ACTGCA GCTCA TCTCA CA CA CA CA
	TCGTTTAACAACTGGAGCTGATGTGAGACATGATATACCAGTGTTGCCAA
	AGCAAATTGTTGACCTCGACTACACTGTACTATATGGTCACAACGGTT
251	ACAGAGTTGGTTTGCCTATAAACCAACGGTTTATTTTAGTTGAACTCTCA
	TGTCTCAACCAAACGGATATTTGGTTGCCAAATAAAATCAACTTGAGAGT
301	AATCATGCAGAGCTTTCTGTTACATTAGCGCTGGATGTCACCAATGCATA
	TTAGTACGTCTCGAAAGACAATGTAATCGCGACCTACAGTGGTTACGTAT
351	TGTGGTCGGCTACCGTGCTGGAAATAGCGCATATTTCTTTC
	ACACCAGCCGATGGCACGACCTTTATCGCGTATAAAGAAAG
401	ATCAGGAAGATGCAGAAGCAATCACTCATCTTTTCACTGATGTTCAAAAT
	TAGTCCTTCTACGTCTTCGTTAGTGAGTAGAAAAGTGACTACAAGTTTTA
451	CGATATACATTCGCCTTTGGTGGTAATTATGATAGACTTGAACAACTTGC
	GCTATATGTAAGCGGAAACCACCATTAATACTATCTGAACTTGTTGAACG
501	TGGTAATCTGAGAGAAATATCGAGTTGGGAAATGGTCCACTAGAGGAGG
	ACCATTAGACTCTCTTTTATAGCTCAACCCTTTACCAGGTGATCTCCTCC
551	
33T	CTATCTCAGCGCTTTATTATTACAGTACTGGTGGCACTCAGCTTCCAACT
	GATAGAGTCGCGAAATAATAATGTCATGACCACCGTGAGTCGAAGGTTGA
601	
001	
	GACCGAGCAAGGAAATATTAAACGTAGGTTTACTAAAGTCTTCGTCGTTC
651	
	TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT
701	
	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
751	CTTTCCACTGCAATTCAACACTCTAACAACAACAA
	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAATGAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
	TO TO THE TOTAL TENT TO THE TE
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
	THE STANSFILL TAKET CACACATGCTACACTCAT
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
901	TCGTCACAGTTTCGTCCGAAGCCACAGCAATTTTTTTGGACTTATGAATGC
	AGCAGTGTCAAAGCAGGCTTCGGTGTCGTTAAAAAACCTGAATACTTACG
054	MC Paragraph and a second and a
221	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	The second of th

# FIGURE 3D (CONT'D)

	ACTACAMACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	CCT) C) CTCCCCC
****	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTACGACGT CCATGTCAGGCCCTCAGATACACTACGACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
	TATACCCIATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTAGATCAGAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
3201	TEN CA CERCUATION OF THE PROPERTY OF THE PROPE
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTTCTTTACACAACCACACACAC
	AATAATACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
•	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GALCGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	ACCOMPANIES OF THE PROPERTY OF
ナポンエ	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	THE COORDINATION OF THE CONTROL OF THE COORDINATION OF THE COORDIN
1501	CAAAACCGAGATAATTCCCTTACAACTC
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	TO THE TOTAL TANGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGACACCGGGACGTAGGAGACCGGTTGCTACA
1601	TCA ACA AMCAMOCA A COLORES
	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	TAMACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCCGACCCTTTA A CARACTE
	CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
	TOTALAGAAATGGGAGAGGT
1/01	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA
1751	CTCTTCCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
	CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	THE TAX OF THE PROPERTY OF THE
1801	GGACATTGTAAATTTTCTAACTCAAACTCAAAC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
7054	TGCAG
7027	ACGTC

### FIGURE 4A pAP-144 template primer Ricin-109Eco primer MMP7-1 signal peptide linker A chain B chain primer 1729C Xba primer MMP7-2 PCR mutagenesis PCR mutagenesis gel purify gel purify **EcoRI** Xbal gel purify gel purify MMP-7 linker -EcoRI pBluescript SK B chain A chain Xbal Signal pepude LECORI/ Xbal EcoRI pAP-217 gel purify vector triple ligation

GURE 4B

WT preproricin linker

5'- TTGTGGCGAAGTTTTAATGCTGATGTT-3' -tctttgcttataaggccagtggtgccaaattttaat--agaaacgaatattccggtcaccacggttaaaattaprimer MMP7-1 3' - AGTGTCAAAAGAAACGCAGGTGACCGTI-5'

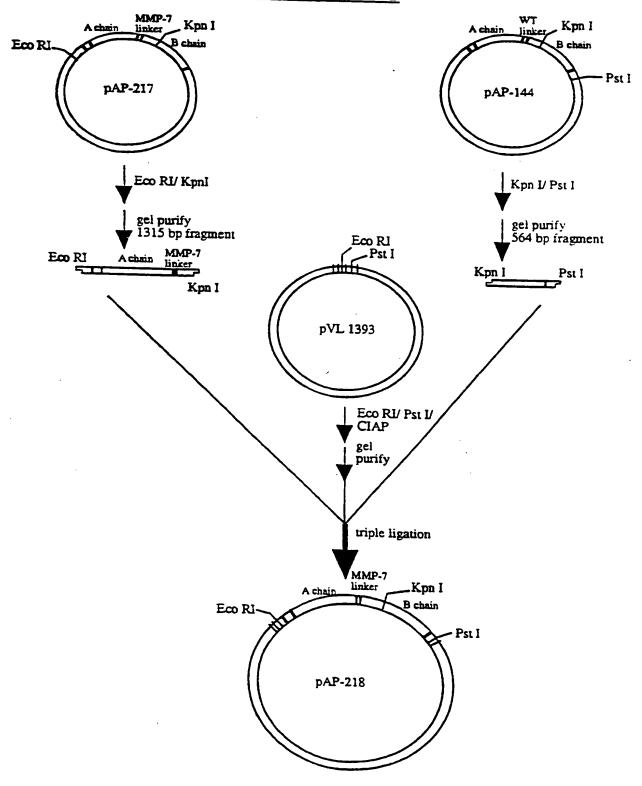
PCR mutagenesis
Ligate with pBluescript SK

pAP 217 linker (MMP-7 variant) --- TCTTTGCGTCCACTGGCATTGTGGCGAAGTTTTAAT -------- AGAAACGCAGGTGACCGTAACACCGCTTCAAAATTA ----

primer MMP7-2

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## FIGURE 4C



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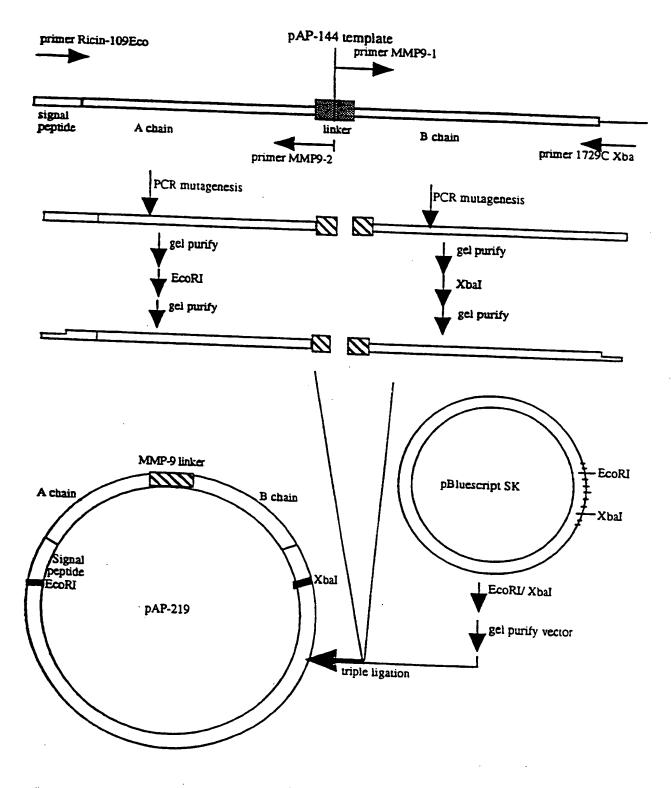
# FIGURE 4D

		.10	20	30	)	40	50
1	GAATTCA CTTAAGT	 TGAAAC: ACTTTG:	CGGGAGGAAA CCCTCCTTT	TACTATT	GTAATAT		- 1
51	GGCNNCN	MCCC		VIGWIWN	CATTATA	CCTACA:	IACGTCA
			IGTTTTGGAT ACAAAACCTA	GGIGGAC	TCCCACC	'Agaaag'	TGTAATC
101	AGGATAA TCCTATT	CAACAT! GTTGTA!	ATTCCCCAAA IAAGGGGTTT	CAATACO GTTATGO	CAATTAT GTTAATA	AAACTT TTTGAA	TACCACA
151	GCGGGTG	CCACTG	rgcaaagcta Acgtttcgat	<b>~</b> • • • • • • • • • • • • • • • • • • •			
201	TCGTTTA	ACAACTY	CACCTC A TO	TC> C> C>			
			COMETAC	WC 1 C 1 G 1	ACTATAT	GGTCAC	<i><b>AACGGTT</b></i>
251	ACAGAGT TGTCTCA	TGGTTT( ACCAAA	SCCTATAAAC CGGATATTTG	CAACGGT GTTGCCA	TTATTT AAAATAA	AGTTGAJ TCAACT	ACTCTCA IGAGAGT
301	AATCATG	CAGAGO	ے دستان کی کا انتظام				
•				TWYTCGC	GACCTAC	agtggt:	TACGTAT
351	ACACCAG	GGCTAC( CCGATG(	CGTGCTGGAA CACGACCTT	ATAGCGC	ATATTTC	TTTCAT	CTGACA
401	ATCAGGA	AGATGC	CA ACCA AMO				
	TAGTCCT	TCTACG:	AGAAGCAATC PCTTCGTTAG	IGAGTAG	TTTTCAC AAAAGTG	TGATGTT ACTACAI	TAAAAT ATTTTD
451	CGATATA	CATTCG	CTTTGGTGG: GAAACCACC				
501	TGGTAAT	CTGAGAC	ים מתחת ממבב				
				r CHACCC	TTTACCA	GGTGAT	TCCTCC
551			TTATTATTACI \ATAATAATG	CVICYC	CACCGTG	<b>AGTCGA</b>	GGTTGA
601	CIGGCIC	GTTCCTT	TTATAATTTG( \ATATTAAAC(				
651	ATTCCAA!	TATATY	AGGGAGAAA? TTTTTTTTTTTTTTT				
701	GATCTGC	ACCAGA1	CCTACCCTA		CTCTTAA	TCCATGI	TGGCCT
751					MACTUTT	ATCAACC	CCCTCT
	GAAAGGTY		TCAAGACTC LAGTTCTCAGA	21 100 1 1	CCTCGGA	AACGATC	AGGTTA
801	TCAACTG	CAAAGAC	GTAATGGTT( CATTACCAA(				
851	TATTAAT	CCTATO	CATAGCTCTCI STATCGAGAGC				
	TCGTCAC	אייידיערט			TATCTAC	GCGTGGA	GCTGGT
				GWCCGI	AACACCG(	CTTCAAA	ATTACC
	TOWIGIAL.	IGTATG(	SATCCTGAGC	CATAGT	GCGTATC	STAGGTO	GAAATG

# FIGURE 4D (CONT'D)

	ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	${\tt GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT}$
1051	${\tt CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA}$
1101	${\tt GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACGCTTTCTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGCCTTTCACAAAATTGATGAATGA$
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	${\tt ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCCTGACTACGGGGGGGG$
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACACGTCTAGATCAGATCAAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACTAATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG ACGTC

22/254 **FIGURE 5A** 



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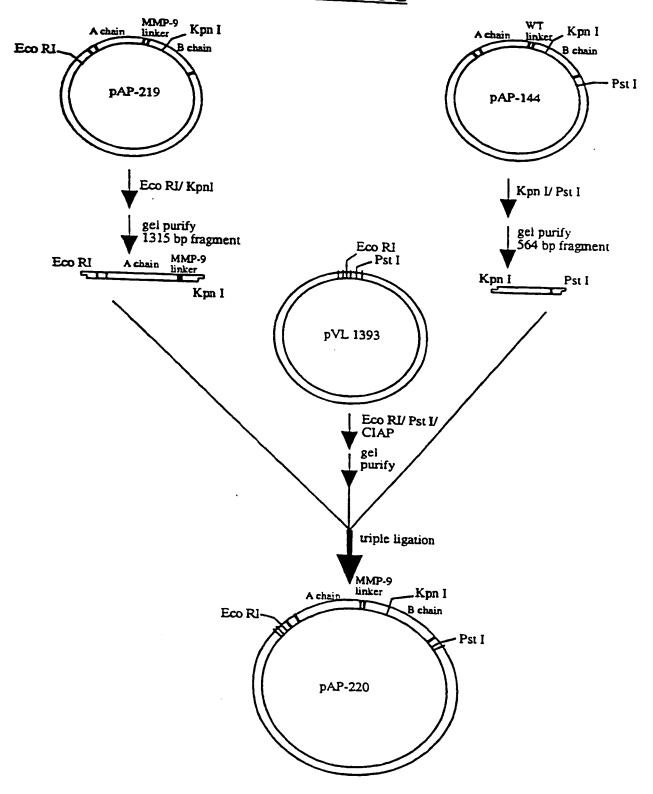
# IGURE 51

primer MMP9-1	5'- GGGCAGCGAAATTTTAATGCTGAT -3'  TCTTTGCTTATAAGGCCAGTGCCAAATTTTAAT  AGAAACGAATATTCCGGTCACCACGGTTTAAAATTA  ** *** *** ***  3'- AGCAGTGTCAAAAGAGGCGTTCCTTAACGT-5'	primer MMP9-1	PCR mutagenesis	ligate with pBluescript SK	pAP 219 linker (MMP-9 variant)	TCTCCGCAAGGAATTGCAGGGCAGCGAAATTTTAAT———TCTCCGCAAGGGAATTTTAAT———AGAGGCGTTCCTTAACGTCCCGTCGCTTTAAAATTA
	3'- AGCAGTGTCAA					

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# FIGURE 5C



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## FIGURE 5D

	10	20	30	40	50
1	GAATTCATGAAACCO CTTAAGTACTTTGGO	GGAGGAAA CCCTCCTTT	 PACTATTGTAA ATGATAACATT	TATGGATGTA ATACCTACAT	TGCAGT ACGTCA
51	GGCAACATGGCTTT( CCGTTGTACCGAAA	TTTTGGAT(	CCACCTCAGGG GGTGGAGTCCC	TGGTCTTTCA ACCAGAAAGT	CATTAG GTAATC
101	AGGATAACAACATA?	PTCCCCAAA!	CAATACCCAAT	TATAAACTTT	ACCACA
	TCCTATTGTTGTATA	AAGGGGTTY	STTATGGGTTA	ATATTTGAAA	TGGTGT
151	GCGGGTGCCACTGTC	GCAAAGCTA	CACAAACTTTA	TCAGAGCTGT	TCGCGG
	CGCCCACGGTGACAC	CGTTTCGAT	GTGTTTGAAAT	AGTCTCGACA	AGCGCC
201	TCGTTTAACAACTGC	SAGCTGATG	rgagacatgat	TATACCAGTGT	TGCCAA
	AGCAAATTGTTGACC	CTCGACTAC	Actctgtacta	ACACTGGTATA	ACGGTT
251	ACAGAGTTGGTTTGG	CCTATAAAC	CAACGGTTTAT	TTTAGTTGAA	CTCTCA
	TGTCTCAACCAAACG	GGATATTTG	GTTGCCAAATA	AAATCAACTT	GAGAGT
301	AATCATGCAGAGCTTAGTACGTCTCGA	rtctgttac. Nagacaatg	ATTAGCGCTGC	ATGTCACCAA TAGAGTGGTT	TGCATA ACGTAT
351	TGTGGTCGGCTACCO	STGCTGGAA	ATAGCGCATAT	TTCTTTCATC	CTGACA
	ACACCAGCCGATGG	CACGACCTT	IATCGCGTATA	DATDAAAAAAA	GACTGT
401	ATCAGGAAGATGCAG	GAAGCAATC	ACTCATCTTT	CACTGATGTT	CAAAAT
	TAGTCCTTCTACGTG	CTTCGTTAG	IGAGTAGAAA	AACAACTACAA	GTTTTA
451	CGATATACATTCGCC	CTTTGGTGG	TAATTATGATA	GACTTGAACA	ACTTGC
	GCTATATGTAAGCGC	GAAACCACC	ATTAATACTAT	CTGAACTTGT	TGAACG
501	TGGTAATCTGAGAGA ACCATTAGACTCTC	AAAATATCG. PTTTATAGC	AGTTGGGAAAT TTTTTTTAATT	OKTOKOOTOO?	AGGAGG TCCTCC
551	CTATCTCAGCGCTT	TATTATTAC.	AGTACTGGTGG	CACTCAGCTT	CCAACT
	GATAGAGTCGCGAA	ATAATAATG	TCATGACCACC	CGTGAGTCGAA	GGTTGA
601	CTGGCTCGTTCCTT	TATAATTTG	CATCCAAATGA	TTTCAGAAGC	AGCAAG
	GACCGAGCAAGGAA	ATATTAAAC	GTAGGTTTACT	AAAGTCTTCG	TCGTTC
651	ATTCCAATATATTG:	AGGGAGAAA	TGCGCACGAG!	ATTAGGTACA	ACCGGA
	TAAGGTTATATAAC	TCCCTCTTT	ACGCGTGCTCT	TAATCCATGT	TGGCCT
701	GATCTGCACCAGAT	CCTAGCGTA	ATTACACTTGA	GAATAGTTGG	GGGAGA
	CTAGACGTGGTCTA	GGATCGCAT	TAATGTGAACT	CTTATCAACC	CCCTCT
751	CTTTCCACTGCAAT GAAAGGTGACGTTA	TCAAGAGTC AGTTCTCAG	TAACCAAGGA( TACCTTGGTTA	CCTTTGCTAG	TCCAAT AGGTTA
801	TCAACTGCAAAGAC AGTTGACGTTTCTG	GTAATGGTT CATTACCAA	CCAAATTCAG? GGTTTAAGTC!	TGTGTACGATG	TGAGTA ACTCAT
851	TATTAATCCCTATC ATAATTAGGGATAG	ATAGETETE	ATCCTCTATA	73 moogas	
901	TCGTCACAGTTTTC	TCCGCAAGG	AATTGCAGGG(	CAGCGAAATTI	TAATGC
	AGCAGTGTCAAAAG	AGGCGTTCC	TTAACGTCCC(	GTCGCTTTAAA	ATTACG

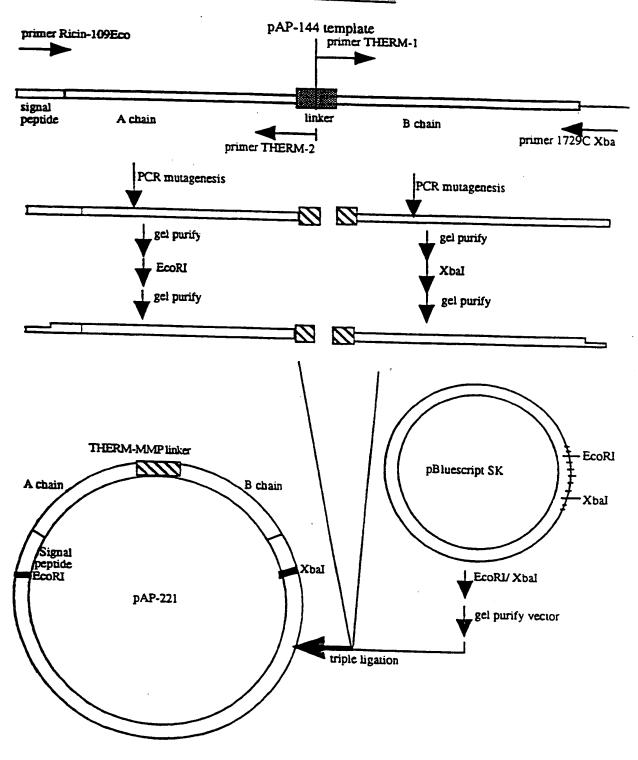
#### SUBSTITUTE SHEET (RULE 26)

# FIGURE 5D (CONT'D)

	E SE (CONT D)
95:	L TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACCATATCGTAGGTCGAAATG
	ACTACAAACATACCTAGGACCCATAGTGCGTATCGTAGGTCGAAATG
	TO THE PROPERTY OF THE PROPERT
1001	GTCTATGTGTTGATGTTAGGGATGGAACATTCCA
	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTACATA
	TO TAMOGIGINATION OF THE PROPERTY OF THE PROPE
1051	CAGTTGTGGCCARCONACTOR
	GTCAACACCGGTA CCTTCA CAMAIACAGATGCAAATCAGCTCTGGACTTT
	TALGITTAGTCACACACACACACACACACACACACACACACACAC
1101	GAAAAGAGACAATACTATTATTATTATTATTATTATTATTATTATTAT
	CTTTTCTCTCTCTACTACTACTACTACTACTACTACTACT
	THE PROPERTY OF THE PROPERTY O
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAGCTGCA
	CCATGTCAGGCCCAGGCTATGTGATGATTGTATGATTGCAATACTCCAGGCCAGGCCAGGCCAGGCCCAGGCCCAGGCCAGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGGCCAGAGCCAGAGCAGAGCCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGAGAGCAGAGAGCAGAGAGCAG
	CCATGTCAGGCCCTCAGATACACTACTAGATACTGCAATACTGCTGCA
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTATTACCCTATTACCTATTACCCTATTACCCTATTACCCTATTACCCTATTACCTATTACCTATTACCTATTACCTATTACCTATTACCTATTACCTATACC
	TCI CTI CCACCCGCTGGCAAATATGGGATAATGGAACCATGA
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1751	CACATTAGG
1201	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC GTCTAGATCAGATC
	GTCTAGATCAGATCAAAATCGTCGCTGTACTACTACAGAACAGTGGTACCACAC
	GTCTAGATCAGAAAATCGTCGCTGTAGTCCCTTGTCACCACAC
1301	TTACAGTGCAAACAACAACAACAACAACAACAACAACAACAACAACA
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCOOMOOOOOO
	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATACTCCACACACACACACACACACACAC
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACACCCCCCCCCCCCCCCCCCCCCCCCCC
	TCCGACTTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
1501	CAAAACCGAGATAATTACCCCCCCCCCCCCCCCCCCCCC
	GTTTTGGCTCTATTA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGCCCTGCATCCTCTGGCCAACGATCCATCC
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACGTTAGAT
	AGTTCTT AT GGAACCATTTAAATTTGTATAGTGGATTCCTCTT
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTTAGTAACCATCCCA
	CATTCCCATCCGAGCCTTAAACAAATCATTCTTTAACAAATCATTCTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAAATCATTCTTTTAACAATCAATCATTCTTTTAACAAT
	CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTTAGAAAATGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTATACCAATGGTAATAAAAACCAGATTACT
• •	ACCIONACCAAACCAAATATGGTTACCATTATTTTCATACATA
	ACCACTGGGTTTGGTTTATACCAATGGTAATAAAAGAGATTACT
	CACALITA AGING TO TO TO TO TO THE TOTAL TO T
	GAGAACGTCACACACAGGACGGTACTTTTATCTACCGAATTTATTT
-501	
	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
	TGCAG
-001	IGCAG
	ACGTC

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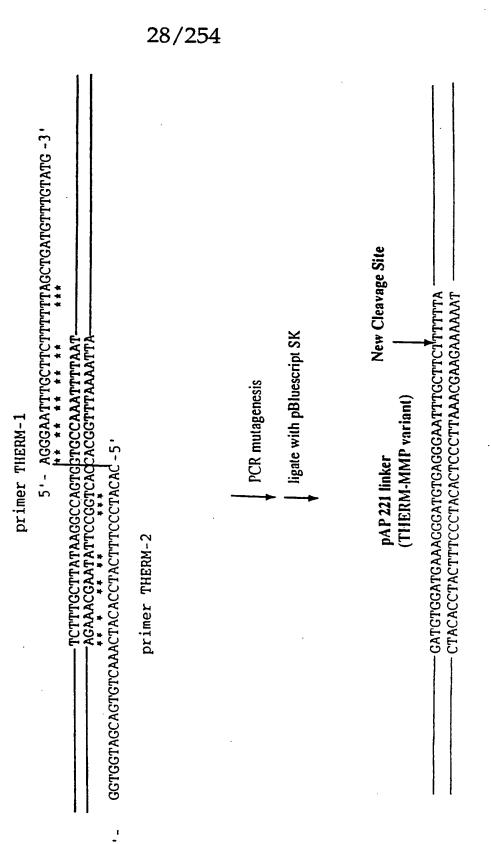
## FIGURE 6A



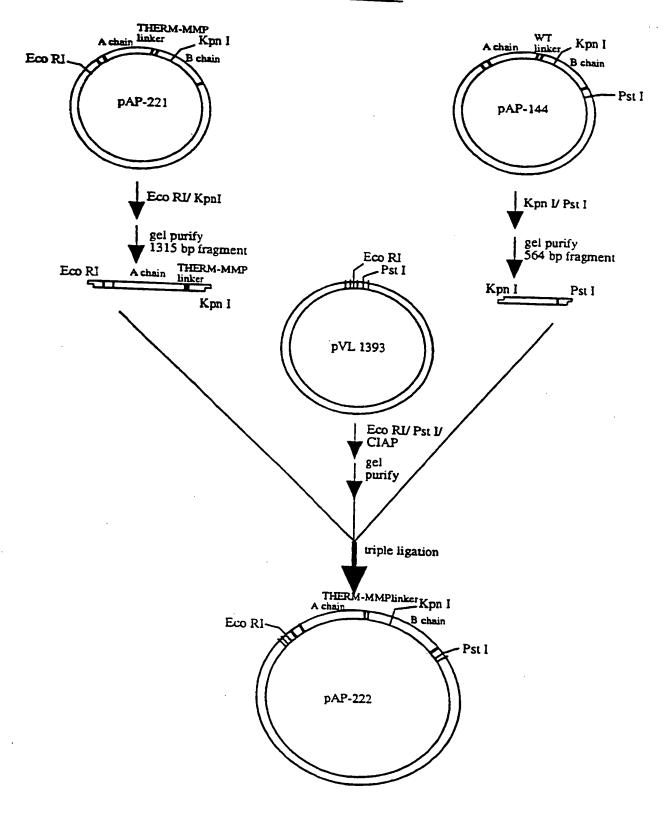
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# IGURE 6B

WT preproricin linker



## FIGURE 6C



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# FIGURE 6D

	10	2	0	30	40	
1	GAATTCATGA CTTAAGTACT	AACCGGGAG	 Gaaatact		-1	50   
			ANIGA	TANK CALLA	ATACCTAC:	<b>LTACGTCA</b>
51	GGCAACATGG CCGTTGTACC	CTTTGTTTT GAAACAAAA	GGATCCAC CCTAGGTC	CTCAGGG? GAGTCCCI	CCAGAAA	CACATTAG
101	AGGATAACAA TCCTATTGTT	רבידה מידובים	~~~~			
151	GCGGGTGCCA	ע ע ע היהודינה				
201			-0117.01.01	TIGNAATI	<i>\GTCTCGA</i> C	AAGCGCC
	AGCAAATTGT			IGIACIAI	ATGGTCAC	AACGGTT
251	ACAGAGTTGG TGTCTCAACC	س لا شارات کیلیزیل				
301	AATCATGCAG	<b>すじしむむむしかい</b>	TOTA			
351				CGCCACCA	ACAGTGGI	<b>TACGTAT</b>
221	TGTGGTCGGC ACACCAGCCG	TACCGTGCT( ATGGCACGA(	GGÄAATAG CCTTTATC	CGCATATI GCGTATA	TCTTTCAT	CCTGACA
401	ATCAGGAAGA	TCCACAACC				
			- ING LUNG	THGHANAG	TGACTACA	AGTTTTA
#21	CGATATACAT GCTATATGTA		mic CLITIM	WINCIATO	TGAACTTG	TTGAACG
501	TGGTAATCTG. ACCATTAGAC	ACACAAAA	. maa			
551	CTATCTCAGC	ه منس لانتمامات	nm			
601	CTGGCTCGTT( GACCGAGCAA	ته لا لا له لا بالمحليات	MM			
651	ATTCCAATAT	TO THE STATE OF TH		GIIIACTA	AAGTCTTC	GICGIIC
				arec reful	aatccatg'	TTGGCCT
701	GATCTGCACCI CTAGACGTGG	ACD TOOMS OF				
751	CTTTCCACTGG GAAAGGTGACG	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~				
801	TCAACTGCAA	AGACGTD 2 700				
				TAMOTUAC.	ACATGCTA(	CACTCAT
	TATTAATCCC:			CATATCT.	ACGCGTGG:	AGGTGGT
901	TCGTCACAGT: AGCAGTGTCAL	TTG A TCTCC	ma			
951	TTTAGCTGAT	STTTGTATGG	ATCCTGA	CCCATAG	IGCGTATC(	STAGGTC

#### SUBSTITUTE SHEET (RULE 26)

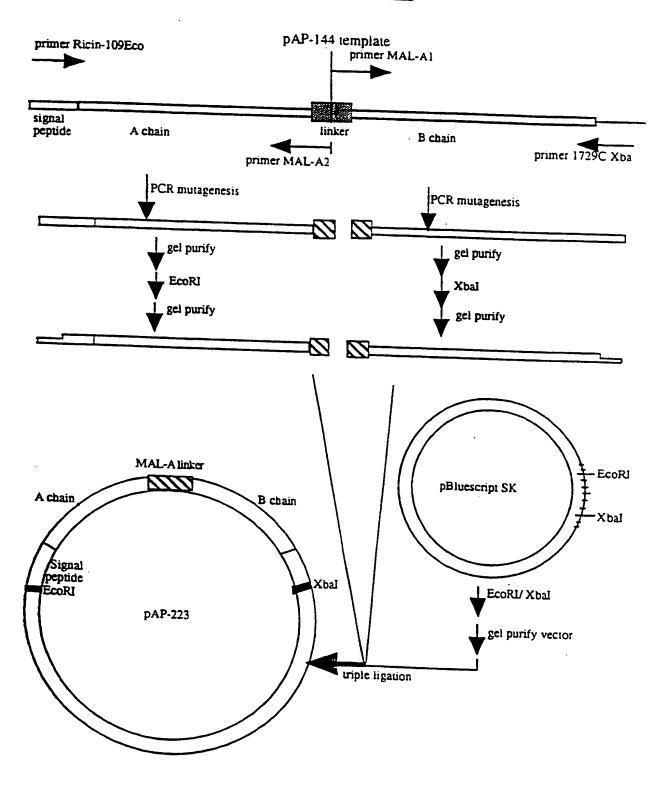
# FIGURE 6D (CONT'D)

	AAATCGACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAG
1001	GAAATGGTCTATGTTGATGTTAGGGATGGAAGATTCCACAACGGAAAC
1051	THE TAXABLE PROPERTY OF THE PR
1051	GCAATACAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTG CGTTATGTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGAC
1101	CACTUMEN AND COMPANY OF THE PROPERTY OF THE PR
	GACTTTGAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTA CTGAAACTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGAT
1151	
	CTTACGGGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACT GAATGCCCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGA
1201	GCTGCAACTGATGCCACCCCCTCCCAAATTATTCCCAAATTATTCCCAAAATTCCCAAATTCCCAAAATTCCCAAATTCCCAAAAATTCCCAAAAATTCCCAAAAATTCCCAAAAATTCCCAAAAATTCCCAAAAATTCCCAAAAATTCCCAAAAAA
	CGACGTTGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTA
1251	AAATCCCAGATCTAGTCTAGTTTTACCAGGGGGGGGGGG
	TTTAGGGTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCAT
1301	CCACACTTACAGTGCAAACCAACATTTATTATTCCCCTT
	GGTGTGAATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAA
1351	CCTACTAATAATACACAACCTTTTCTTACAACCA
	GGATGATTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACC
1401	TCTGTGCTTGCAAGCAAATAGTCGACAACTA TCGACAACTA
	AGACACGAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGT
1451	GTGAAAAGGCTGAACAACAGCCCCCCCCCCCCCCCCCCC
	THE TOTAL CONTROL OF THE TRANSPORT OF TH
1501	CCTCAGCAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGA
	TATACGGAATGTTCACTAAGATTATATGCCCT
1551	AACAGTTGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGAT
	TO THE STANDARD AND THE STANDARD STANDA
1601	GGATGTTCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTG CCTACAAGTTCTTACTACCTTACTTAAATTTGTATAGTGGATTGGTG
	TARCATATCA CATATCA CATATCA CATATCA CCAC
1651	TTAGATGTGAGGGGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCC
	THE TABLE TRANSPORT OF THE TRANSPARTIGES
1701	TCTCCATGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAG
	THE TOTAL TRANSPORT OF THE TRANSPORT OF THE TOTAL TRANSPORT OF THE TOTAL TRANSPORT OF THE TRANSPORT OF THE TOTAL T
1751	ATTACTCTCTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAA
	THE TOTAL PROPERTY OF THE TAXABLE PROPERTY.
1801	TAAAAAGGACATTGTAAATTTTCTAACTCAACTCAACTC
	THEACATIGACTTTCCTGTCAATATAGC
1851	AATTCCTGCAG TTAAGGACGTC

WO 98/49311 PCT/CA98/00394

32/254

## FIGURE 7A



SUBSTITUTE SHEET (RULE 26)

# IGURE 7B

WT preproricin linker

primer MAL-A1

5'- AATTATGATGAAGAGGATGCTGATGTTTGTATG -3' ·TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT--AGAAACGAATATTCCGTGACCACGGTTTAAAATTA-3'- GGTAGCAGTGTCAAAGTCCACCAAGTTAACGTC-5"

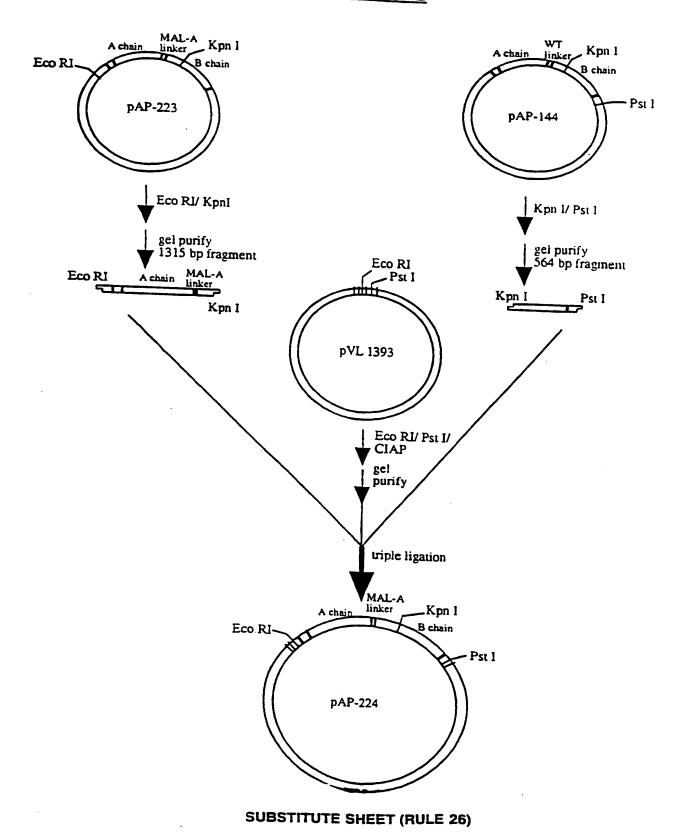
primer MAL-A2

PCR mutagenesis

Iigate with pBluescript SK

pAP 223 linker (MAL-A variant) 

## FIGURE 7C



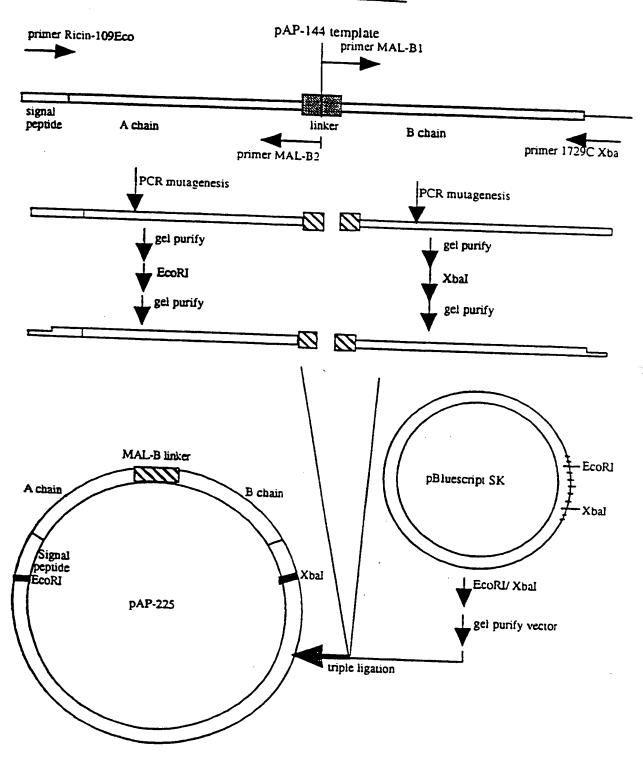
## FIGURE 7D

	10	20	30	40	50
1	GAATTCATGAAACO	I CGGGAGGAAA GCCCTCCTTT	ractattgta Atgataacat	 ATATGGATGT; TATACCTACA'	ATGCAGT IACGTCA
51	GGCAACATGGCTT:	rgttttggat	CCACCTCAGG	GTGGTCTTTC.	ACATTAG
	CCGTTGTACCGAA	Acaaaccta	GGTGGAGTCC	CACCAGAAAG	TGTAATC
101	AGGATAACAACAT	ATTCCCCAAA	CAATACCCAA	TTATAAACTT	TACCACA
	TCCTATTGTTGTA	IAAGGGGTTT	GTTATGGGTT	AATATTTGAA	ATGGTGT
151	GCGGGTGCCACTG'	TGCAAAGCTA	CACAAACTTT	ATCAGAGCTG	TTCGCGG
	CGCCCACGGTGAC	ACGTTTCGAT	GTGTTTGAAA	TAGTCTCGAC	AAGCGCC
201	TCGTTTAACAACT	GGAGCTGATG	TGAGACATGA	TATACCAGTG	TTGCCAA
	AGCAAATTGTTGA	CCTCGACTAC	ACTCTGTACT	ATATGGTCAC	AACGGTT
251	ACAGAGTTGGTTT	GCCTATAAAC	CAACGGTTTA	TTTTAGTTGA	ACTCTCA
	TGTCTCAACCAAA	CGGATATTTG	GTTGCCAAAT	AAAATCAACT	TGAGAGT
301	AATCATGCAGAGC	TTTCTGTTAC	ATTAGCGCTG	GATGTCACCA	ATGCATA
	TTAGTACGTCTCG	AAAGACAATG	TAATCGCGAC	CTACAGTGGT	TACGTAT
351	TGTGGTCGGCTAC	CGTGCTGGAA	ATAGCGCATA	TTTCTTTCAT	CCTGACA
	ACACCAGCCGATG	GCACGACCTT	TATCGCGTAT	AAAGAAAGTA	GGACTGT
401	ATCAGGAAGATGC	AGAAGCAATC	ACTCATCTTT	TCACTGATGT	TCAAAAT
	TAGTCCTTCTACG	TCTTCGTTAG	TGAGTAGAAA	AGTGACTACA	ACTTTTA
451	CGATATACATTCG	CCTTTGGTGG	TAATTATGAT	AGACTTGAAC	AACTTGC
	GCTATATGTAAGC	GGAAACCACC	ATTAATACTA	TCTGAACTTG	TTGAACG
501	TGGTAATCTGAGA ACCATTAGACTCT	GAAAATATCG	AGTTGGGA A A	<b>TGGTCC3 CT3</b>	C) CO) CO
551	CTATCTCAGCGCT	OATTATTATT	AGTACTGGTG	GCACTCAGCT	TCCAACT
	GATAGAGTCGCGA	DTAATAATAA	TCATGACCAC	CGTGAGTCGA	AGGTTGA
601	CTGGCTCGTTCCT	OTTTAATATT	CATCCAAATG	ATTTCAGAAG	CAGCAAG
	GACCGAGCAAGGA	OAAATTATAA	GTAGGTTTAC	TAAAGTCTTC	GTCGTTC
651	ATTCCAATATATT	GAGGGAGAAA	TGCGCACGAG	AATTAGGTAC	AACCGGA
	TAAGGTTATATAA	CTCCCTCTTT	ACGCGTGCTC	TTAATCCATG	TTGGCCT
701	GATCTGCACCAGA CTAGACGTGGTCT	TCCTAGCGTA YAGGATCGCAT	ATTACACTTG	AGAATAGTTG	GGGGAGA TOTOOOO
751	CTTTCCACTGCAA GAAAGGTGACGTT	TTCAAGAGTC AAGTTCTCAG	TAACCAAGGA ATTGGTTCCT	GCCTTTGCTA	GTCCAAT CATDDAC
801	TCAACTGCAAAGA AGTTGACGTTTCT	CGTAATGGTT		- - - - -	CMC 3 CM 3
851	TATTAATCCCTAT	CATAGCTCTC	ATGGTGTA TI	\	W001 001
901	TCGTCACAGTTTC AGCAGTGTCAAAC	AGGTGGTTC	א מייניים בא אייניים א		

# FIGURE 7D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC GTCTAGATCAGATC
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTATACCAATGGTAATAAAACTATCTGTCTAATGA
1751	CTCTTGCAGTGTGTGTCTCCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG ACGTC

# FIGURE 8A



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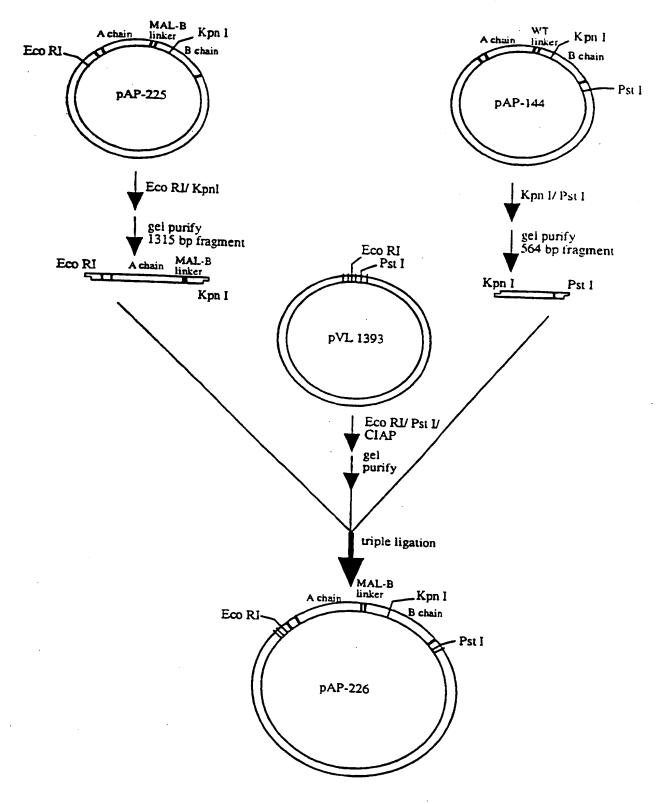
FIGURE 8

WT preproricin linker

Primer MAL-B1  5'- TCGGAGGACAATGATGATGTTTGTATG -3'  AGAAACGAATATTCGTTCGTGTCCAAATTTTAAT  AGAAACGAATATTCCGGTCACACGGTTTAAATTAT  Primer MAL-B2    Iigalc with pBluescript SK    MAL-B variant)	TTGCCGATTTTCGGGGAATCGGAGACAATGATGAA——————————
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SUBSTITUTE SHEET (RULE 26)

## FIGURE 8C



SUBSTITUTE SHEET (RULE 26)

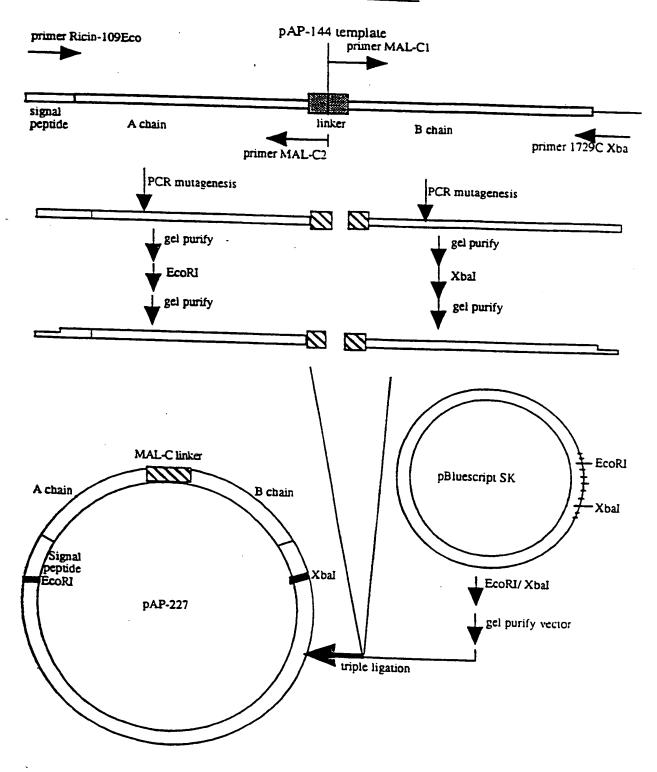
# FIGURE 8D

	10	20	30	40	50
1	GAATTCATGAAAC CTTAAGTACTTTG	 CGGGAGGAAA	 TACTATTGTA:	TATCOL	ī
			VIGYIYYCVI.	PATACCTACAT:	ACGTCA
51	GGCAACATGGCTT	TCTTTTTCCSM	0010		
			ag 1 g C M C 1 C C C	CACCAGAAAGT	STA A TC
101	AGGATAACAACAT TCCTATTGTTGTA	ATTCCCCAAA	CAATACCCAAT	TATAAACTTT	אררארא
			GITAIGGGTT	<b>LATATTTGAAA</b> 1	LCCACA.
T 2 T	GCGGGTGCCACTG CGCCCACGGTGAC	TGCAAAGCTA	CACAAACTTT	TCAGAGCTGT	rcecec
			O.G. I.IGMAM	AGTCTCGACA	GCGCC
	TCGTTTAACAACT AGCAAATTGTTGA	GGAGCTGATG' CCTCGACTAC	TGAGACATGAT ACTCTCTACTA	ATACCAGTGTT	rgccaa
251	ACAGAGTTCGTTT		rerered MC 12	TATGGTCACA	CGGTT
	ACAGAGTTGGTTT( TGTCTCAACCAAA(	GGATATTTG(	CAACGGTTTAT GTTGCCAAATA	TTTAGTTGAAC	TCTCA
301	AATCATGCAGAGC	T-T-T-C-T-C-T-C-T-T-T-T-T-T-T-T-T-T-T-T		MAATCAACTTC	AGAGT
	AATCATGCAGAGCT TTAGTACGTCTCG	AAGACAATG	ATTAGCGCTGG PAATCGCGACC	ATGTCACCAAT	GCATA
351	TGTGGTCGGCTAC	CTCCTCC			
	TGTGGTCGGCTACC ACACCAGCCGATGC	CACGACCTT	NAGCGCATAT NATCGCGTATA	TTCTTTCATCC	TGACA
401	ATCAGGAAGATGC	GAACCAAMO			
	TAGTCCTTCTACG	CTTCGTTAGT	GAGTAGAAAA	CACTGATGTTC GTGACTACAAC	TAAAA
451	CGATATACATTCCC	CTTTCCMCC			
			er enverage LWL	CTGAACTTCTT	CAACC
501	TGGTAATCTGAGAG				
			CONCCCT LIM	CCAGGTGAでつか	
551	CTATCTCAGCGCTT	مت حصص و بناس و بارد			
			CYTONCCMCC	GTGAGTCCAAG	COMPAN N
POI	CTGGCTCGTTCCTT GACCGAGCAAGGAA	TATAATTTGC	ATCCAAATGA	TTTCAGAAGCA	GCAAG
			TAGGITIACT	AAAGTCTTCCT	
031	ATTCCAATATATTG TAAGGTTATATAAC	AGGGAGAAAT	GCGCACGAGA	ATTAGGTACAA	CCGGA
			eccorec ICT	LAATCCATCTY	
	GATCTGCACCAGAT CTAGACGTGGTCTA	CCTAGCGTAA GGATCGCATT	TTACACTTGA(	BAATAGTTGGG	GGAGA
	CTTTCCACTGCAAT	TCNNCN		-TTATCAACCC	CCTCT
	CTTTCCACTGCAAT GAAAGGTGACGTTA	AGTTCTCAGA	AACCAAGGAG( TTGGTTCCTC	CTTTGCTAGT	CCAAT
801	TCAACTGCAAAGAC	CTA A MOCESTA			
	AGTTGACGTTTCTG	CATTACCAAG	CAAATTCAGT( GTTTAAGTCA(	TGTACGATGT(	GAGTA
851	TATTAATCCCTATC	ATACCMON			
			CCVCVTVTC	CACGCGTGGAG	
901	TCGTCACAGTTTTT	CCCC3 =====			
	AGCAGTGTCAAAAA	CGGCTAAAAG	CCCCTTAGCCT	CCTGTTACTAC	AAGC TTCG

## FIGURE 8D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC GTCTAGATCAGATC
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	
1601	
1651	
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTTTTTT
1751	
1801	
1851	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG TGCAG

## FIGURE 9A



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# IGURE 9

WT preproricin linker

primer MAL-C1

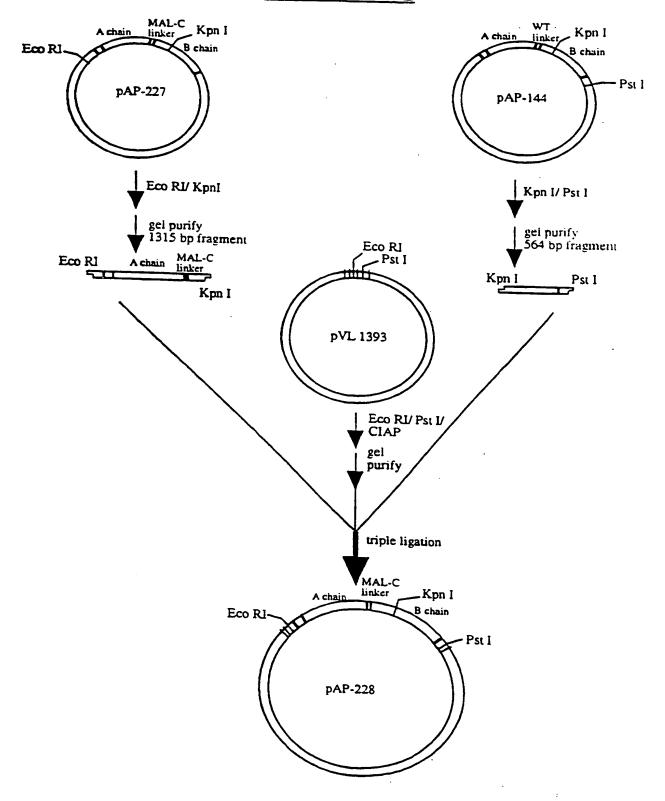
5 - GCGATATCAGTTACTATGGCTGATGTTTGTATG -3 tctttgcttataaggccagtgcgaggcaaatttaat-\*agaaacgaatattcggicaccacggttaaaatta-3'- GGTAGCAGTGTCAAAGTCCACCAATGTCCCCTT'-5'

primer MAL-C2

PCR mutagenesis
Ilgate with pBluescript SK

pAP 227 linker (MAL-C variant) SUBSTITUTE SHEET (RULE 26)

## FIGURE 9C



SUBSTITUTE SHEET (RULE 26)

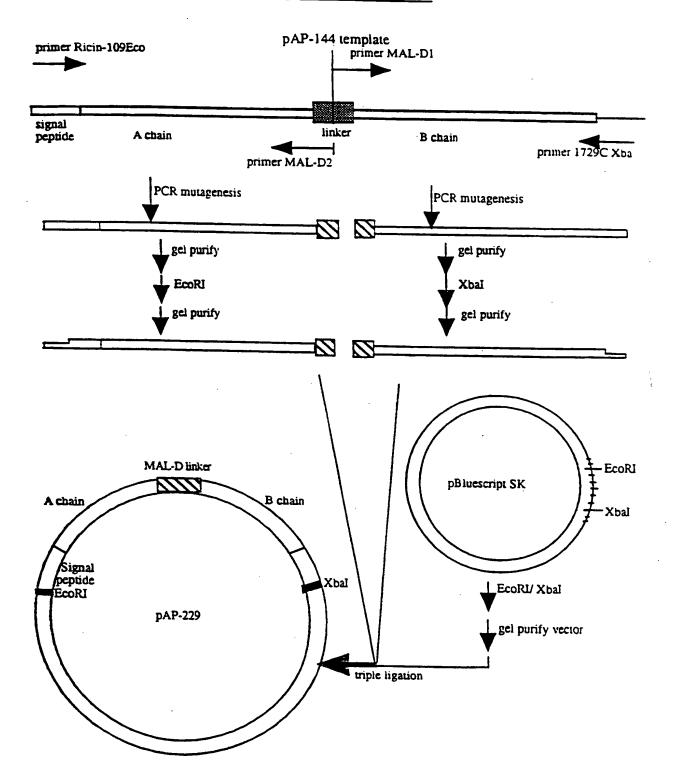
## FIGURE 9D

	1	.0	20	30	4	0 5	0
.1	GAATTCATO CTTAAGTAC	  AAACCGGG  TTTGGCCC	 AGGAAAT TCCTTTA	 ACTATT AGTAA	OTAATATO COATATACO	 ATGTATGCAG TACATACGTC	T A
51	GGCAACATO CCGTTGTAO	GCTTTGTT CGAAACAA	TTGGATO AACCTAG	CACCTC GTGGAG	AGGGTGGTC	TTTCACATTA AAAGTGTAAT	G C
101	AGGATAACI TCCTATTGT	ACATATTO OAATATDT	CCCAAAC GGGTTTC	AATACC TTATGG	CAATTATAS GTTAATATT	ACTTTACCAC TGAAATGGTG	A
151	GCGGGTGCG	ACTGTGCA STGACACGT	AAGCTAC TTCGATC	ACAAAC TGTTTG	TTTATCAGA AAATAGTCI	GCTGTTCGCG CGACAAGCGC	G C
201	AGCAAA11(	TIGACCIC	GACTACA	CTCTGI	ACTATATGO	AGTGTTGCCA TGGCAACACT	T'
251	ACAGAGTTO TGTCTCAAO	GTTTCCC1 CAAACGGA	ATAAACO TATTTGO	CAACGGI STTGCCA	TTATTTTAG AATAAAATO	TTGAACTCTC SAACTTGAGAG	A
301	AATCATGC! TTAGTACG!	GAGCTTTC CTCGAAAC	TGTTACI ACAATG1	ATTAGCO CAATCGC	CTGGATGTC GACCTACAG	ACCAATGCAT ATGCATACGTA	'A T
351	TGTGGTCG(	CTACCGTC CGATGGCAC	CTGGAA! GACCTT	ATAGCGC PATCGCG	ATATTTCTT TATAAAGAA	TCATCCTGAC AGTAGGACTG	A T
401	ATCAGGAA( TAGTCCTTY	SATGCAGA! CTACGTCTT	GCAATC! CGTTAG1	ACTCATO PGAGTAG	TTTTCACTO	ATGTTCAAAA TACAAGTTTI	T. A'
451	CGATATAC:	ATTCGCCTT FAAGCGGAA	TGGTGGT ACCACC	rattaat Xtaatt <i>i</i>	GATAGACTT CTATCTGA	GAACAACTTG LCTTGTTGAAC	iC :G
501	TGGTAATC: ACCATTAG	rgagagaai Actetett	ATATCG:	AGTTGG( CAACC(	SAAATGGTCC TTTACCAGC	CACTAGAGGAG STGATCTCCTC	:G
551	ONINGAGI	CGCGAAAT	MTAATG:	CATGAC	CACCGTGAC	CAGCTTCCAAC GTCGAAGGTTG	A
	ONCCONGC	WOONWY 13	(TTAAAC)	STAGGTT	PTACTAAAG1	AGAAGCAGCAA CCTTCGTCGTT	C
651	ATTCCAAT: TAAGGTTA	ATATTGAG( IATAACTC(	GAGAAA: CTCTTT	TGCGCA( ACGCGT(	CGAGAATTAC CTCTTAATC	GTACAACCGG CATGTTGGCC	A; T
701	GATCTGCA CTAGACGT	CCAGATCC: GGTCTAGG	ragegtai Ategeat	ATTACA( TAATGT(	CTTGAGAAT! GAACTCTTA	AGTTGGGGGAG	A T
751	CTTTCCAC GAAAGGTG	TGCAATTC: ACGTTAAG'	AAGAGTC! ITCTCAG	TAACCAI ATTGGT:	AGGAGCCTT:	rgctagtcca; Acgatcaggt	T/ A1
801	TCAACTGC AGTTGACG	AAAGACGT. TTTCTGCA'	AATGGTT CAACCAA	CCAAAT' GGTTTA	TCAGTGTGT: AGTCACACA:	ACGATGTGAGT ACGATGTGAGT	A1 T
851	TATTAATC ATAATTAG	CCTATCAT. GGATAGTA	AGCTCTC TCGAGAG	ATGGTG' TACCAC	TATAGATGC( ATATCTACG(	SCACCTCCAC( CGTGGAGGTG(	CA ST
901	TCGTCACA AGCAGTGT	GTTTCAGG CAAAGTCC	TGGTTAC ACCAATG	AGGGGA	AGCGATATC	AGTTACTATG(	3C

# FIGURE 9D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	THE TANGET AGGAC TOGGG TATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAACTGTAACTAAC
	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATCTCATCATCATCATCATCATCATCATCATCATCAT
	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
	THE TACAL TACTACATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGCTGGCGACCATTATACGGATAATGGAACCATCATAAATCC
	THE PROPERTY OF THE PROPERTY O
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTAGATCAGATCAAAACGCCCCCCCCCCCCCCCCCCC
	GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
	THE STATE OF THE CARLES AND THE CARCEGARGES AND A CONTROL OF THE CARLES AND A CONTROL
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGAAAACAATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCAGTATAGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCG1CTTCTTC1GGC1CTTTATGCAGATGGTTCAATACGTCCTCAG
	TO THE CHARGA AND THE CONTROL OF THE
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	TO THE STATE OF TH
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGAACACGGGACGACGATGGATGT
	ACAATTCTAGGAGAGAACACCGGGACGTAGGACCGTTGCTACCTAC
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	AGTTCTTACTACCTTCCTAAAATTTCTATAGTGGATTGGTGTTAGAT
	TARACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
	CACTCGCTAGCCTAGCCTTAACAAATCATTCTTTACCCTCTCCA
	TOTAL
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA
1751	CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	GAGALGATALALA
	GAGAACGTCACACACAGGACGGTACTTTATCTACCGAATTTATTT
1803	GGACATTCTA AATTTCTA ACTOR
	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG
	ACGTC
	UPAT P

#### FIGURE 10A



SUBSTITUTE SHEET (RULE 26)

# FIGURE 10B

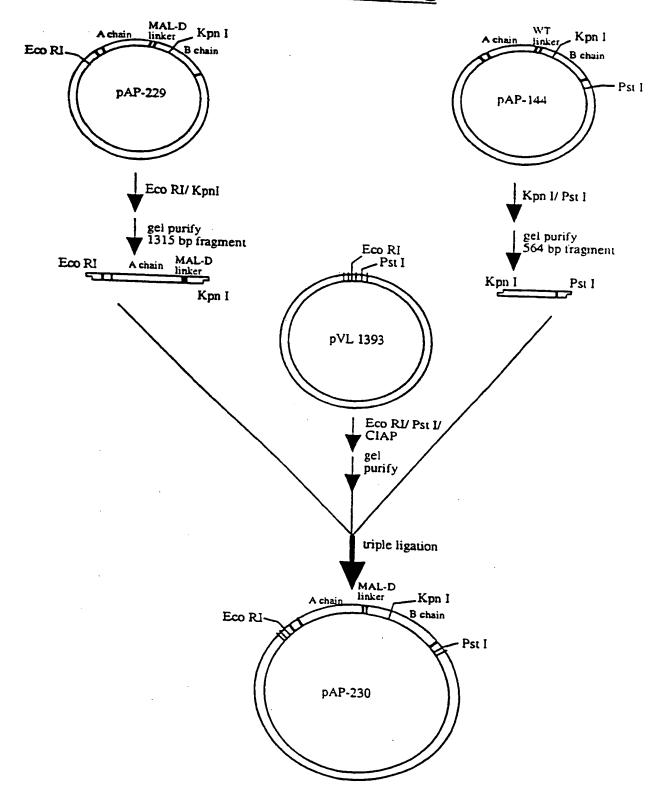
WT preproricin linker

primer MAL-D1

5'- CTGTCGTTCCCTACTAATGCTGATGTTTGT -3' ligate with pBlucscript SK -tctttgcttataaggccagtggtgccacgcaaattttaat--agaaacgaatattcggtgcaccacggttaaaatta-GCTTTGGAGAGGATCCTGTCGTTCCCTACTAAT CGAAACCTCTTGCAAGGACAGCAAGGGATGATTA PCR mutagenesis (MAL-D variant) pAP 229 linker 3'- GGTAGCAGTGTCAAACGAAACCTCTCTTGCAAG<sup>(-5'</sup> primer MAL-D2

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# FIGURE 10C



SUBSTITUTE SHEET (RULE 26)

# FIGURE 10D

		10	20	30	40	
1	C) ) mmos		i	- 1	40	50
_	CTTAAGT	TGAAACCGG ACTTTGGCC	GAGGAAA1 CTCCTTTA	IACTATTGTA TACAATAACAT	ATATGGATG	TATGCAGT
		_		oninchi	INTACCTAC	ATACGTCA
21	GGCAACA	rectitel	TTTGGAT	CACCTCAGG	<b>ら</b> からにかしかかっ	·C > C > C > C
				A TROWGICC	CACCAGAAA	GTGTAATC
101	AGGATAA	CAACATATT				
			00001110	PITATEGGTT	AATATTTGA	AATGGTGT
151	GCGGGTG	CACTGTGC				
	CGCCCAC	GTGACACG	TTTCGATC	ACAAACTTT. TGTTTGAAA	ATCAGAGCT TAGTCTCGA	GTTCGCGG
201	TCGTTTA	ACABOTTON	~~~~			
	AGCAAAT	CTTCACCTOM(	CTGATGT	GAGACATGA	TATACCAGT	GTTGCCAA
			C TWCN	CICICIACT	ATATGGTCA	CAACGGTT
251	ACAGAGT	CGGTTTCCC	TATA A A C C			
	TGTCTCA	ACCAAACGG	ATATTTGG	AACGGTTTA! TTGCCAAAT	TTTAGTTG	AACTCTCA
		*		- 1 account L	MAAATCAAC	TTGAGAGT
301	AATCATGO	CAGAGCTTTC	TGTTACA	TTAGCGCTG		
				waterche (	-TACAGTGG	TTACGTAT
351	TGTGGTC	GCTACCCTV				
	ACACCAGO	CGATGGCAC	GACCTTT	TAGCGCATA! ATCGCGTATI	LITCITTCA	TCCTGACA
401	DTCDCCD >				PARCHAMAGT.	AGGACTGT
401	TACTOCTO	GATGCAGA	GCAATCA	CTCATCTTT	CACTGATG	ת א א א שרידי
				OVG I VGWWY	GIGACTAC	AAGTTTT
451	CGATATAC	ATTCGCCTT	TGGTGGT	AATTATGATA	Cacamora	
				TIMMINCIN	CTGAACTT(	STTGAACG
501	TGGTAATC	TGAGAGAAA	200000			
				CAMCCCCT.T.T.T.	<b>LCCAGGTGA</b>	TCTCCTCC
551	CTATCTCA	GCGCTTTN A	Ma mma			
				GTACTGGTGG CATGACCACC	GIGAGTCG	<b>えみらにアサビァ</b>
601	CIGGCICG	$\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}$	ים במשתח ל לי	•		
	GACCGAGC	AAGGAAATA	TTAAACG	ATCCAAATGA TAGGTTTACT	TTTCAGAA( AAAGTCTT	CAGCAAG
651	ATTCCAAT	ריי איי איי איי איי איי איי איי				-GICGITC
_	TAAGGTTA	TATA I CACC	GAGAAAT	GCGCACGAGA	ATTAGGTAC	CAACCGGA
				-0-0106161	TAATCCATC	TTCCCCM
701	GATCTGCA	CCACATCCT	3000			
	CTAGACGT	GGTCTAGGA	TCGCATT	ITACACTTGA AATGTGAACT	GAATAGTTO AACTATTT	GGGGAGA
751	CTTTCCAC	TCC3 3 TTC3				cccctct.
	GAAAGGTG	ACCTTA ACT	AGAGTCT	AACCAAGGAG	CCTTTGCTA	GTCCAAT
				100116616	GGAAACGAT	د تشتنات لا ب
801	TCAACTGC	みみることででかり	3 MOOSS			
				CAAATTCAGT STTTAAGTCA	CACATGCTA	(C) (C) (C) (C)
851	TATTAATC	ここでな かし かかっし				
	ATAATTAG	GGATAGTAT	GCTCTCAT CGAGAGTI	rggtgtatag Accacatatc	ATGCGCACC	TCCACCA
901	TEGTEREN	CTTTCC				WOOLGGI.
	AGCAGTGT	CAAACCAAA	GGAGAGAI	ACGTTCCTGT FGCAAGGACA	CGTTCCCTA	CTA ATCC
		CGWM	$\sim$	rgcaaggaca	CCARCOS	

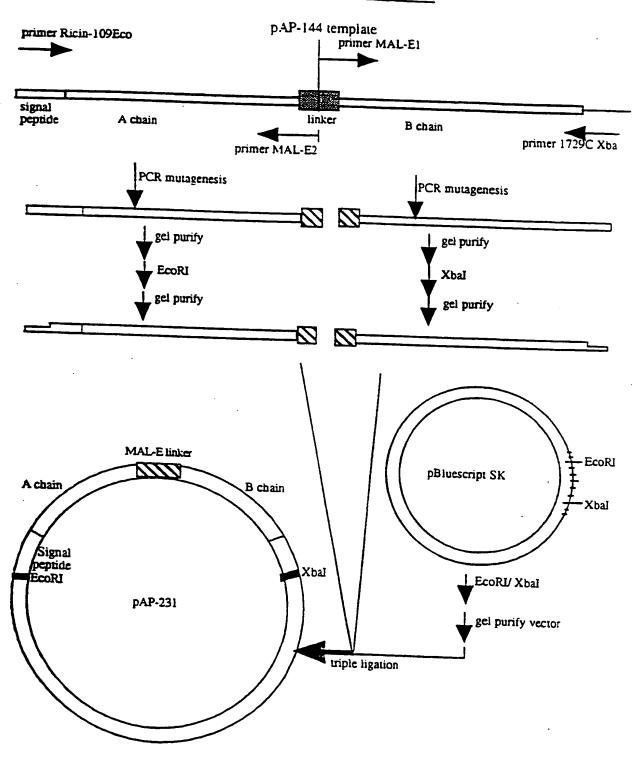
## FIGURE 10D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATCCAAATCAAATCAACCTCCAAATCAACCTCCAAATCAACCTCCAAATCAAATCAACCTCCAAATCAAATCAACCTCCAAATCAACCTCCAAATCAAATCAACCTCCAAATC
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CITTICICIGITATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACACTCTTAGCAGC
	THE TOTAL CASA I CASA I CGT CGCT GT AGT CCCTT GT CACCAT GGT GT G
	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACTAATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAGTCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCCCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG ACGTC

WO 98/49311 PCT/CA98/00394

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## FIGURE 11A



#### SUBSTITUTE SHEET (RULE 26)

IGURE 11B

WT preproricin linker

primer MAL-E1

5'- AATAATTCACAGCATCAGGCTGATGTTTGTATG -3'	TCTTTGCTTATAAGGCCAGTGCCAAATTTTAAT——AGAAAÇGAATATÇCGGTCACACGGTTTAAAATTA——AGAAAÇGAATATÇCGGTCACCACGGTTTAAAATTA	. 5-1
-,5	TCTTTGCTTATAAGGCC/	3'- ccraccacrercaaarrraaggrreraraegar <sup>1-</sup> 5'

primer MAL-E2

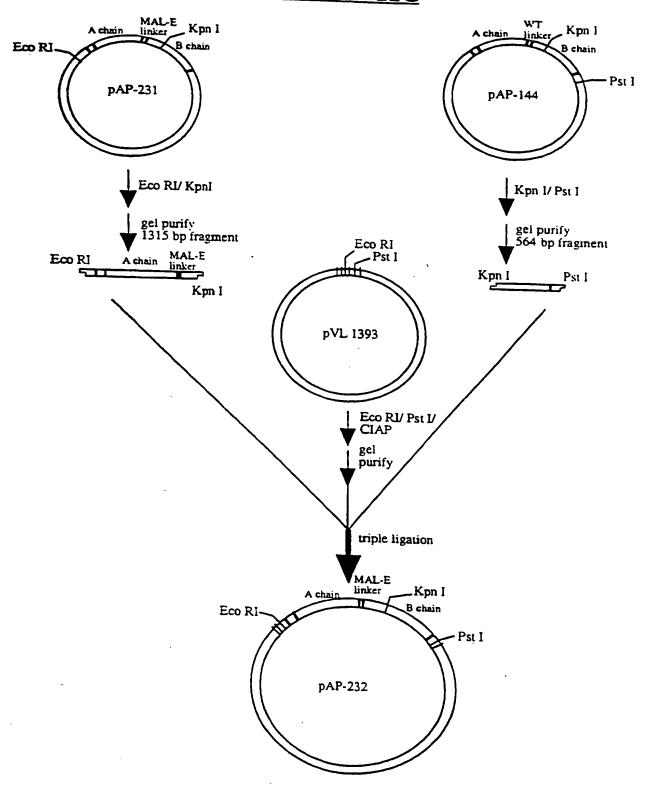
PCR mutagenesis

ligate with pBluescript SK

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pAP 231 linker (MAL-E variant) WO 98/49311 PCT/CA98/00394

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SUBSTITUTE SHEET (RULE 26)

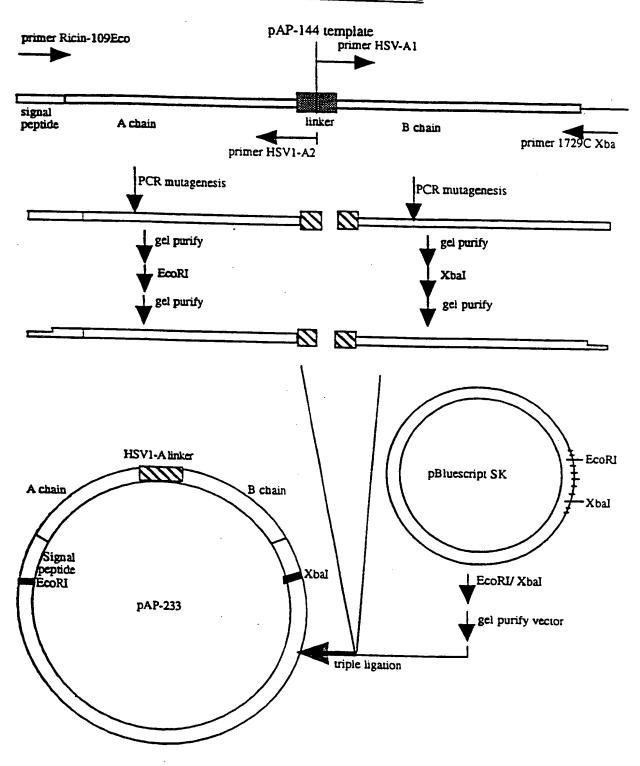
#### FIGURE 11D

	10	20	30	41	50
					ATGTATGCAGT TACATACGTCA
51					TTTCACATTAG AAAGTGTAATC
101					ACTTTACCACA TGAAATGGTGT
151					GCTGTTCGCGG CGACAAGCGCC
201	 	<del>-</del>			AGTGTTGCCAA TCACAACGGTT
251					STTGAACTCTCA CAACTTGAGAGT
301					CACCAATGCATA STGGTTACGTAT
351					ITCATCCTGACA AAGTAGGACTGT
401	 				GATGTTCAAAAT CTACAAGTTTTA
451	 				TGAACAACTTGC ACTTGTTGAACG
501					CACTAGAGGAGG GTGATCTCCTCC
55:	 				CAGCTTCCAACT GTCGAAGGTTGA
60	 				AGAAGCAGCAAG TCTTCGTCGTTC
65					AGGTACAACCGGA CCATGTTGGCCT
70					ragttgggggaga Atcaacccctct
75					TTGCTAGTCCAAT AACGATCAGGTTA
80	 				TACGATGTGAGTA ATGCTACACTCAT
8					CGCACCTCCACCA GCGTGGAGGTGGT
9					CACAGCATCAGGC

# FIGURE 11D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	
1001	GTCTATGTGTTGATGTTAGGGATGGAAGGTTCCACAACGGAAACGCAATA
	CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATCCAACTCTAACTAACTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAACTCTAA
	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	TO THE STATE OF TH
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
	CCATGTCAGGCCTTCAGATGATGATTGCAATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	A CTC A TCCC A CCCCCATA
	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	
1231	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
	-
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
	TO STATE OF THE CARE THE CARCEGARGE ATER
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGLAAACAATGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTCTTCATA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTCTCACCCCCCCCCCCCCCCCCCCCCCCC
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTALCGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTA AGATCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAACACCGGGACGTAGGAGACCGGTTGCTACA
1601	TC 1 1 C 1 TC 1 TC 1 TC 1 TC 1 TC 1 TC
7007	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1681	CROS COOK - CONTROL OF THE CONTROL O
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
	CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
	TAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA
	THE TOTAL STATE AND THE TOTAL STATES AND THE TOTAL
1751	CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	GAGAACGTCACACACACACACACACACACACACACACACA
	TATCTACCGAATTTATTTT
1801	GGACATTGTAAATTTTGTAACTCAAACACA
	CCTGTAACATTTAAAACATTGAAGGACAGGACAGCAAGTTATATCGAATTCC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG
_	ACCTC

#### FIGURE 12A



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TCTGCGCTTGTAAACGCATCGTCGGCACATGTTAATAAGACGCGAACATTTGCGTAGCAGCCGTGTACAATTA

(HSV1-A variant)

# FIGURE 12F

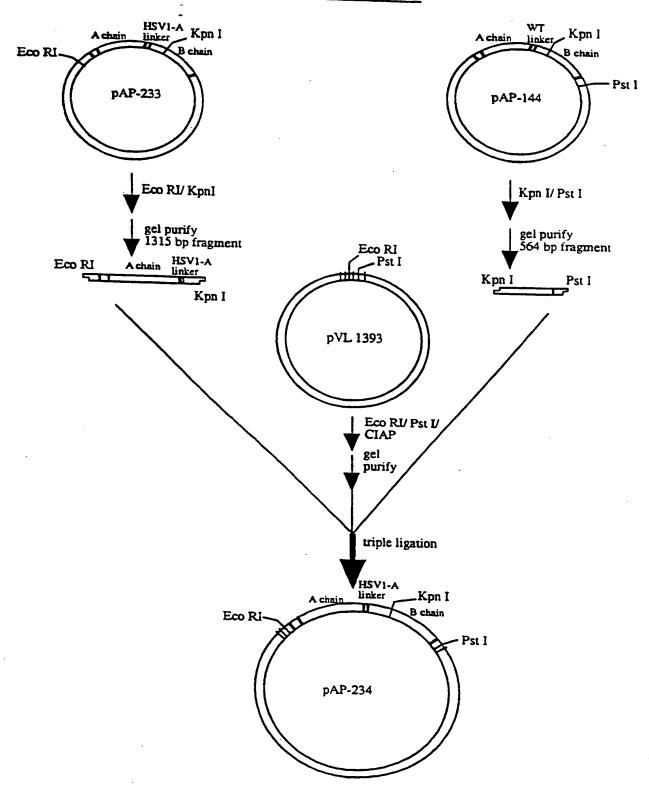
# WT preproricin linker

5'- TÇGTÇGGCAÇATGTTAATGCTGATGTTGT -3' ligate with pBluescript SK ·TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT--AGAAACGAATATTÇÇÇGT|CACCACGGTTTAAAATTA-PCR mutagenesis primer HSV1-A pAP 233 linker 3'- AGCAGTGTCAAAAGACGCGAACATTTGCGT-5 primer HSV1-A

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### FIGURE 12C



SUBSTITUTE SHEET (RULE 26)

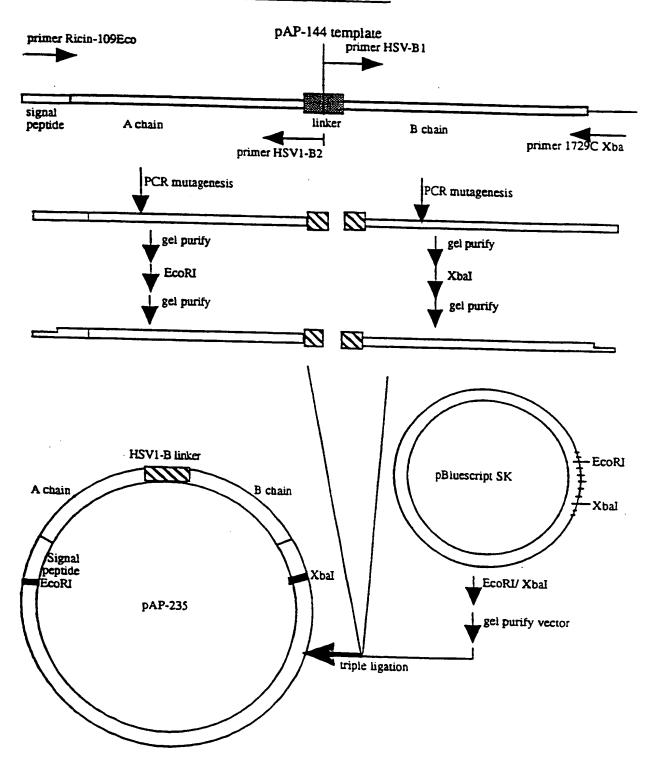
## FIGURE 12D

	10	20	30	40	
1	GA A TOTAL CA TOTAL A S			40	50
-	GAATTCATGAAAC CTTAAGTACTTTC	CGGGAGGAAA	TACTATŤGTA	ATATGGATGTZ	TECNE
			ON INVICAT	TATACCTACAT	'ACCTYC'A
51	GGCAACATGGCTT CCGTTGTACCGA	TGTTTTGGAT	CACCTCACC	CM00====	
	CCGTTGTACCGA	LACAAAACCTA	GTGGAGTCC	CIGGICITICA	CATTAG
101	1001m110			CACCAGAAAGT	GTAATC
101	AGGATAACAACAT TCCTATTGTTGTA	CATTCCCCAAA	CAATACCCAA	ת ממדמרת	
	TCCTATTGTTGT	LTAAGGGGTTT(	STTATGGGTT	AATATTTCA	ACCACA
151	GCGGGTGCCACTA	·maa			TGGTGT
	GCGGGTGCCACTG CGCCCACGGTGAC	ACCEMBAGCTA(	CACAAACTTT	ATCAGAGCTGT	TCCCCC
			OIIIGNAN	TAGTCTCGACA	ACCCCC
201	TCGTTTAACAACT	CC > CC DC >			
	AGCAAATTGTTGA	CCTCGACTAC	CTCTCT	TATACCAGTGT	TGCCAA
			CTCTGTWCT.	ATATGGTCACA	ACCCMM
251	ACAGAGTTGGTTT	CCCMAMA A A			
	TGTCTCAACCAAA	CGGATATTTG	TTGCCD111A	TTTTAGTTGAA	CTCTCA
201	33000			MAAATCAACTT	GAGAGT
301	AATCATGCAGAGC TTAGTACGTCTCG	TTTCTGTTAC	TTAGCGCTG	CATCEC CO.	
	TTAGTACGTCTCG	AAAGACAATGI	AATCGCGAC	CTACACCAA	TGCATA
351	TCTCCTCCTCC			CARCAGIGGTT	ACGTAT
	TGTGGTCGGCTAC ACACCAGCCGATG	CGTGCTGGAAA	TAGCGCATA	ריי ערייהייה ערייה דיים ערייהיים עריים אינים	CMC > c -
	ACACCAGCCGATG	GCACGACCTTI	ATCGCGTAT	AAAGAAAGTAG	CIGACA
401	ATCAGGAAGATCC	3633665			GACIGI
	ATCAGGAAGATGC TAGTCCTTCTACG	MGAAGCAATCA TCTTCCTTT	CTCATCTTT	TCACTGATGTT	TAAAAT
			OUG TACKAN	AGTGACTACA A	
451	CGATATACATTCC				
	GCTATATGTAAGC	GGAAACCACCA	AATTATGATI	AGACTTGAACA	ACTTGC
			WHINCING	「CTGAACTTCT	707700
501	1GGTAATCTCACA		_		
	ACCATTAGACTCT	CTTTTATAGCT	CAACCCTTTT	GGTCCACTAG	AGGAGG
551	CM3 man			CCAGGTGATC	TCCTCC
221	CTATCTCAGCGCT GATAGAGTCGCGA	TTATTATTACA	GTACTGGTGG		
	GATAGAGTCGCGA	aataataatgt	CATGACCACC	GTGACTCAGCTT	CAACT
601	СТСССТССТТССТ			O. O. O. O. C. C. C.	GTTGA
	CTGGCTCGTTCCT GACCGAGCAAGGA	TATAATTTGC	ATCCAAATGA	TTTCAGAACC	
				AAAGTCTTCC	
651	ATTCCAATATATTC TAAGGTTATATAAC	~2CCC2	_		CGIIC
	TAAGGTTATATAA	PROGGAGAAAT	GCGCACGAGA	ATTAGGTACAZ	CCGC
				ייריביתי ביון אא.	$\sim$
701	GATCTGCACCACAC	TCCT1	_		
	CTAGACGTGGTCT	GCATCCCATAA	TTACACTTGA	GAATAGTTGGG	GGAGA
				אר מיזיים וויוווו	`~~~~
751	CTTTCCACTGC N No	PTC 2 2 C 2 C			
	GAAAGGTGACGTT	AGTTCTCAGA	TTGGTTCCRC	CCTTTGCTAGI	CCAAT
				GUAAACCESTOS	~~~~
POT	- LAACTGCAAACA				
	AGTTGACGTTTCTC	CATTACCAAG	STTTAAGTCA	CACATCCATGT	GAGTA
851	TATTAATCOCA			CATGCTACA	CTCAT
	TATTAATCCCTATC	ATAGCTCTCA:	<b>IGGTGTATAG</b>	ATGCGCACCTC	CACCE
				TACCCCCCCA	~~~~
901	TCGTCACAGTTTTC AGCAGTGTCAAAAC	TCCCCCCCC		CGAG	G1001.
	AGCAGTGTCAAAA	ACCCC TTGTA	ACGCATCGT	CGGCACATGTT	'AATCC
			こよびこじましてア		

# FIGURE 12D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	THE TALL AND THE TAGGACT COORDINATE CATACOLAR
1001	GTCTATGTGTTGATGTTACCCATCCAACCAACCAACCAAC
	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CACTUCTOCOCATICOCAT
	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	THE TOTAL OF THE T
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	THE TAXABLE PAGE TAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATCTCATCATCATCATCATCATCATCATCATCATCAT
	GGTACAGTCCGGGAGTCTATGTGATGATGATTGCAATACTGCTGCA
	THE THE TACK THE TACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGCTCGCCCA CCCTTTA TO GGGATAATGGAACCATCATAAATCC
	THE TAXABLE OF TAXABLE O
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	THE TOTAL CONTROL OF THE TRANSPORT OF THE TOTAL CONTROL OF THE TOTAL CON
1301	TTACAGTGCAAACCAACATTTATCCCCTTA
	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
	TO THE STATE OF TH
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
	TO THE TOTAL CONTROL OF THE TO
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
	TATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	THE TAXABLE TAXABLE TAXABLE CONTINUES OF TAXABLE CO
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGAACACCGGGACGTAGGACGGTTGCTACACA
3 - 0 -	
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
	CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTATAGAAATGGGAGAGGT
	THE TANGAMAT GGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA
	THE TAXABLE TO THE TOTAL
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	GAGAACGTCACACACAGGACGGTACTTTATCTACCGAATTTATTT
	TATELLATION OF THE PROPERTY OF
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
	TOTAL TECHNICATION OF THE T
	TGCAG
	ACGTC

#### FIGURE 13A



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TCTACGTATTTACAGGCATCGGAGAAATTTAAGAATAGAATAGTCGTAGCCTCTTTAAATTTAAATTCTTA

(HSV1-B variant)

FIGURE 13E

WT preprorich linker

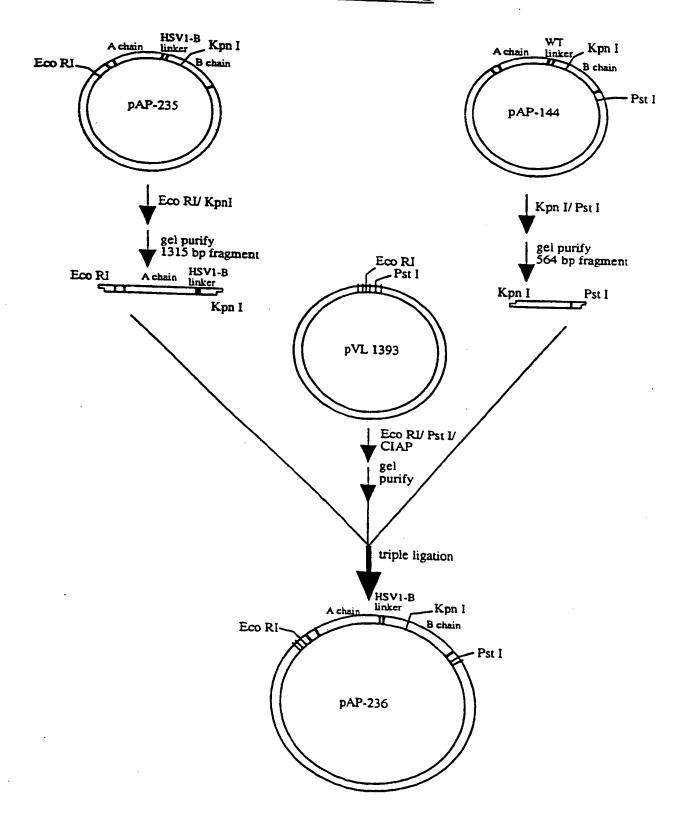
primer HSV1-B

5'- TCGGAGAATTTAAGAATGCTGATGTTTGT ligate with pBluescript SK PCR mutagenesis pAP 235 linker 3'- AGCAGTGTCAAAAGATGCATAAATGTCCGf-5' primer HSV1-B

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#### FIGURE 13D

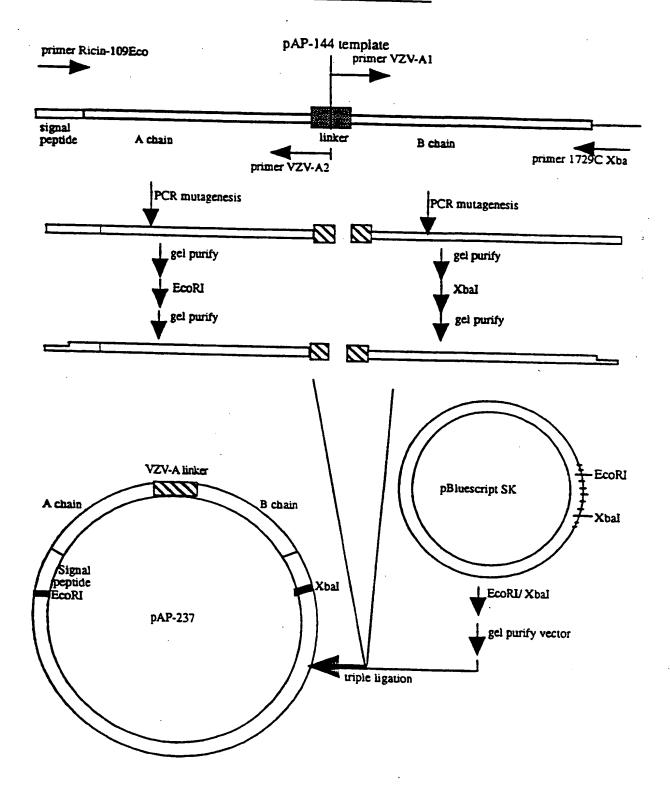
	10	20	30	40	50
1	GAATTCATGAAACO	GGGAGGAAAT	מתבאדת מתראמי ו	1	
	CTTAAGTACTTTG	CCCTCCTTTA	TGATAACAT	TATACCTACA	TACGTCA
51	GGCAACATGGCTT	GTTTTGGATC	CACCTCAGG	GTGGTCTTTC	ACATTAG
	CCGTTGTVCCGVV	CAAAACCTAG	GTGGAGTCC	CACCAGAAAG	TGTAATC
	AGGATAACAACATA TCCTATTGTTGTA	AAGGGGTTTG	TTATGGGTT	aatatttgaa	atggtgt
151	GCGGGTGCCACTG? CGCCCACGGTGAC	CAAAGCTAC	ACAAACTTT	ATCAGAGCTG	TTCGCGG
201	TCGTTTAACAACTC				
	The state of the s	CICGACIACA	CTCTGTACT	ATATGGTCAC	AACGGTT
251	ACAGAGTTGGTTTC	CCTATAAACC	AACGGTTTA!	TTTTAGTTGA	ACTCTCA
รกา	TGTCTCAACCAAAC				
	AATCATGCAGAGCT TTAGTACGTCTCGA	MAGACAATGI	AATCGCGAC	CTACAGTGGT	TACGTAT
351	TGTGGTCGGCTACO ACACCAGCCGATGO	CGTGCTGGAAA CCACGACCTTT	TAGCGCATA!	TTTCTTTCAT	CCTGACA
401	ATCAGGAAGATGC	GAAGCAATCA		701 0-01	
451	TAGTCCTTCTACGT				
	CGATATACATTCGC GCTATATGTAAGCC	GAAACCACCA	'AATTATGAT! .TTAATACTA'	AGACTTGAAC. PCTGAACTTG	AACTTGC TTGAACG
501	TGGTAATCTGAGAG	AAAATATCCA	CTTCCC		
		ININGC1	CAACCCTTT	ACCAGGTGAT	CTCCTCC
	CTATCTCAGCGCTT GATAGAGTCGCGAI	mrwrw1G1	CATGACCAC	CGTGAGTCGA	aggttga
601	CIGGCICGITCCIT	TATAATTTCC	ים מו מוש מו		
		MINI INMACC	TAGGTTTACT	PAAAGTCTTC(	STCGTTC
921	ATTCCAATATATTC TAAGGTTATATAAC	AGGGAGAAAT TCCCTCTTTA	GCGCACGAG CGCGTGCTC	AATTAGGTAC	AACCGGA
701	GATCTGCACCAGAT	CCTAGCGTAA	TTACACMMC		
		GONICGCATI	AATGTGAAC	rcttatcaac(	CCCTCT
751	CTTTCCACTGCAAT GAAAGGTGACGTTI	TTCAAGAGTCT AAGTTCTCAGA	AACCAAGGA TTGTTTGTT	CCTTTGCTA CGGAAACGAT	GTCCAAT CAGGTTA
801	TCAACTGCAAAGAC	GTAATGGTTG	·C		
		INCCMM	GITTAAGTC	ACACATGCTA(	CACTCAT
851	TATTAATCCCTATC ATAATTAGGGATAC	ATAGCTCTCA TATCGAGAGT	TGGTGTATA ACCACATAT	SATGCGCACC CTACGCGTGG	TCCACCA AGGTGGT
901	TCGTCACAGTTTTC	TACGTATTTA	CAGGCATCG	GAGAAATTTA	AGAATGC

# FIGURE 13D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	ACTACA A SOLAT GOAT CC TGAGCCCATAGTGCGTATCGTAGGTCGA A ATC
	ACTACAAACATACCTAGGACTCGGGTATCACGTAGGTCGAAATG
	THE CARLETTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACACAACGCAACGCAACGCAACGCAATA
	CAGATACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
3053	Characteristics
TOST	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
	TOTAL TARGET TAGE TO A LANGE TO THE TERM OF THE TAGE TO A LANGE TO THE TAGE TO
1101	Change
7701	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CTTTTCTCTGTTATGATAACCTACATTTACG
	THE TACE IT CACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTAGTTACTA
	COLDENS TO THE COLD AND COLD A
	CLATGTCAGGCCCTCAGATACTACTAGATACTAGATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTACGATACGCCACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCGTATACATCATAAATCC
	TEACTA CONTROL INGCAAATATGGGATAATGGAACCATCATA A ATCC
	TGACTACGGTGGCCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
	THE CITEGIAGIA TIAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC GTCTAGATCAGATC
	GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTGTGT
	TOTAL CARACTEGET CONTROL CONTR
	The state of the s
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
	11GG11G1AATACGCCAATCAGTTCCAACCGAAGGATCA
1251	2. The contract of the contrac
TOOT	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTCTTA
	TTATTATGTGTTGGAAACAATGTTGTTGTTGTGTGTGTGT
	TO THE PROPERTY OF THE PROPERT
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTTATAGAGGACTGTAGCAGTGAAA
T401	CI IGCAAGCAAATAGTGGACAAGTATGGATAGACCACTCTA
	GAACGTTCGTTTATCACCTCTTCATACCAGAGACTGTAGCAGTGAAA
	TOTAL CONTROL OF THE PROPERTY
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAATACGTCCTCAG
	TOCAL CHARGACAGA TGGCTCTTTATGCAGATGGTTCAATACCTCCTCA
	TCCACTTGTTGTCACCCGAGAAATACGTCTACCAACTTCTACGTCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTCCCTTT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	TIGGETETAT TAACGGAATGTTCACTAAGATTATATCCCCTTTCCCCTTTCCCCTTTCCCCTTTCCCCCTTTCCCC
3	THE
T221	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGACACCGGGACGTAGGAGGCCGGTTGCTACCTAC
	ONGRANCA CCGGGACGTAGGAGACCGGTTGCTACCTACTACTACTACTACTACTACTACTACTACTACTA
1601	MOSSOCIACIACA
TOOT	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACTTTAAACTTGAT
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
	TO THE PROPERTY OF THE PROPERT
1651	GTGAGGCGATCGGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTCTTTACCCTCTCCA
TODI	GIGAGGCGATCGGATCCGAGCCTTAAACAAATCAATCA
	CACTCCGCTAGCCTAGGCTTAAACAAATCATTCTTTACCCTCTCCA
	TIGHTAGAAATGGGAGAGAGA
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
	16G1GACCCAAACCAAATATGGTTACCATTATTTTCATA
	ACCACTGGGTTTGGTTTATACCAATCCTTATACT
•	TO THE PROPERTY OF THE PROPERT
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	CICI GEAGIGIGIGIGICCTGCCATGAAAATACATGCCCCCCCCCC
	GAGAACGTCACACACACAGGACGCTACTTTTTTTTTTTT
	TO THE PROPERTY OF THE PROPERT
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTCTCTCTCTCTC
	COTOTA A CARMANT I TOTAACTGAAAGGACAGCAAGTTA TATOCA A MINO
	COLGIANCATTTAAAACATTGACTTTCCTGTCCTTCAAATTCC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG
	ACGTC
	·

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#### FIGURE 14A



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# FIGURE 14B

# WT preproricin linker

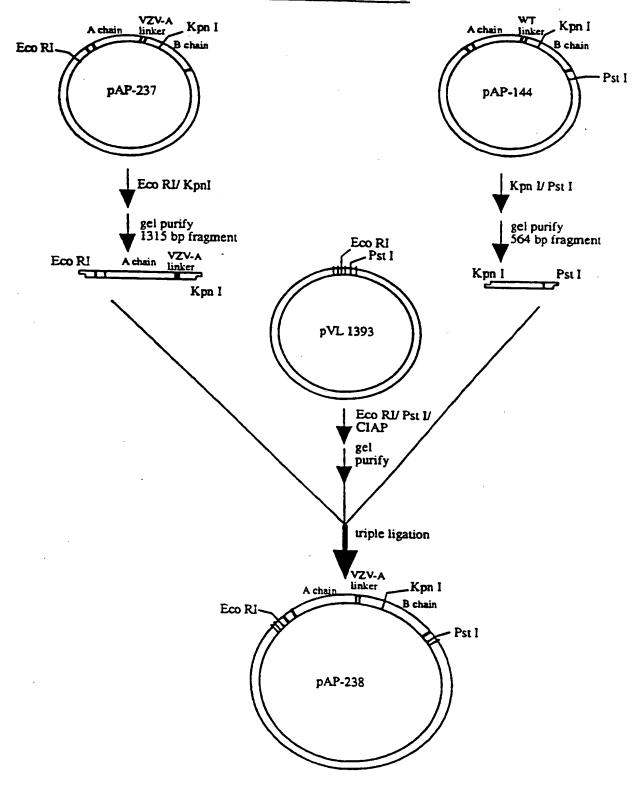
primer VZV-A1

5'- GTGGAGGCAAGTTCTAATGCTGATGTTTGT ligate with pBluescript SK -tctttgcttataaggcca<mark>stggtgccaaattttaat</mark> -agaaacgaatattçççgifcaccacggtttaaaatta-- TCTCAGGATGTAAAÇGCAGTGGAGGCAAGTTCTAAT-AGAGTCCTACATTTGCGTCACCTCCGTTCAAGATTA PCR mutagenesis (VZV-A variant) pAP 237 linker 3' - AGCAGTGTCAAAAGAGTCCTACATTTGCGT-5' primer VZV-A2

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#### FIGURE 14C



SUBSTITUTE SHEET (RULE 26)

# FIGURE 14D

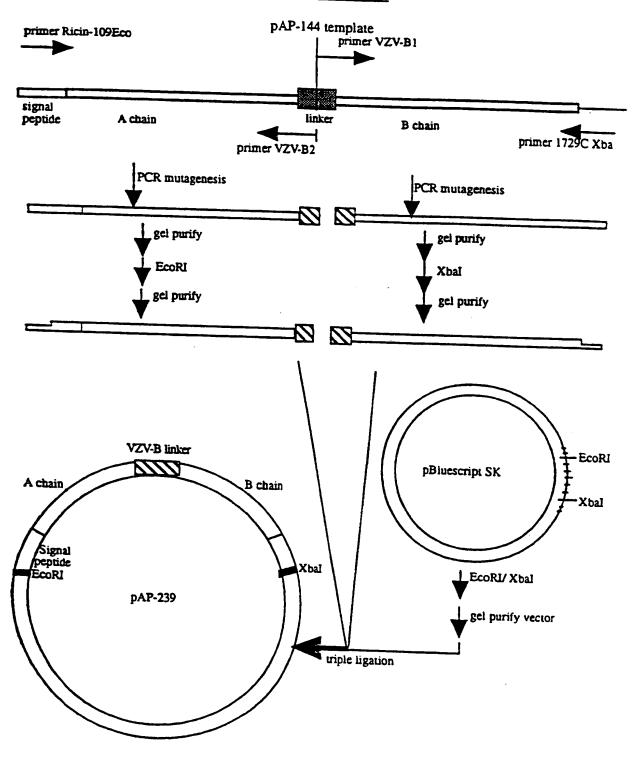
	10	20		30	40	50
1	GAATTCATGA	AACCGGGAC	~ ~ ~ ~ ~			
	GAATTCATGA CTTAAGTACT	11000000	TTTATG	ATAACA?	TATACCTA	CATACGTCA
51	GGCAACATGG	CTTTGTTTT	GATCCA	CCTCAGO	CTCCTCT	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
			-CIAGGT	GGAGTC	CACCAGAA	<i>AGTGTAATC</i>
101	AGGATAACAA	CATATTCCC	CAAACAA	ת מררים ו	ስጥጥ አጥ አ አ <b>አ</b> ረ	
		GINIMOGGG	31-1-1-G-1-T	ATGGGT.	PARTATTTO	<b>Aaatggtgt</b>
151	GCGGGTGCCA	CTGTGCAAA	CTACAC	AAACTT	PATCAGAGO	مات سميد المحادث
		ONCACGIII(	-GATGTG	TTTGAA	ATAGTCTCG	BACAAGCGCC
201	TCGTTTAACA	ACTGGAGCT	SATGTGA	GACATG	TATACCAC	
		TONCETEGAL	TACACT	CTGTAC	PATATGGTC	ACAACGGTT
251	ACAGAGTIGG	TTTGCCTATA	AACCAA	CGGTTT	TTTTAGTT	CARCTOTON
	TGTCTCAACC	AAACGGATAT	TTGGTT	GCCAAAT	PAAAATCAA	CTTGAGAGT
301						
	AATCATGCAG TTAGTACGTC	AGCT I TCTGT TCGAAAGACI	LATGTAA	AGCGCT( TCGCGA(	GATGTCAC CTACAGTG	CAATGCATA GTTACGTAT
351	TGTGGTCGGC	TACCGTGCTC	GAAATA	GCGC A TO		1 DOODA
		A I GOCACGA(	CTTAN	CGCGTAT	DAAAGAAAG	TAGGACTGT
401	ATCAGGAAGA TAGTCCTTCT	TGCAGAAGC	LATCACTY	CATCTT	יי ער ארייט איי	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
		Medici ico	INGTGA	STAGAAA	LAGTGACTA	CAAGITITA
451	CGATATACAT	<b>LCCCCLLLCC</b>	TGGTAA!	TTATGAT	AGACTTGA	ACAACTTGC
		AGCGGAAAACC	ACCATT	AATACTA	<b>TCTGAACT</b>	TGTTGAACG
501	TGGTAATCTG	<u>A</u> GAGAAAAT;	TCGAGT.	TGGGAAA	TGGTCCAC	TAGAGGAGG
E C 1			AGCTCA	ACCCTT	CACCAGGTG	ATCTCCTCC
22I	CTATCTCAGC	GCTTTATTAT	TACAGT	ACTGGTG	GCACTCAG	CTTCCAACT
		-0,52,137,13	MIGICA:	IGACCAC	CGTGAGTC	GAAGGTTGA
POT	CTGGCTCGTT	CCTTTATAA1	TTGCAT	CAAATO	SATTTCAGA	AGCAGCAAG
651		OCHUINI	MACG TA	GTTTAC	TAAAGTCT	TCGTCGTTC
021	ATTCCAATAT TAAGGTTATA	ATTGAGGGAG	AAATGC	GCACGAC	SAATTAGGT	ACAACCGGA
			- I I I MCG	CTGCTC	TTAATCCA	TGTTGGCCT
701	GATCTGCACC	AGATCCTAGO	יתיים מ מידים:	. ~ . ~ ~ ~		
	CTAGACGTGG	TCTAGGATCO	CATTAN	PGTGD AC	AGAATAGT	TGGGGGAGA
					-ICITATCA	ACCCCCTCT
751	CTTTCCACTG	CAATTCAAGA	GTCTAA	CAAGGA	GCCTTTGC	TAGTCCAAT
			CHONII	act.t.c.c.l	CGGAAACG	ATCAGGTTA
801	TCAACTGCAA AGTTGACGTT	AGACGTAATO	GTTCCA	AATTCAC	TGTGTACG	ATGTGAGTA
			CWGG I	TAAGTC	CACACATGO	TACACTCAT
851	TATTAATCCC	TATCATAGCT	CTCATG	STGTATA	GATGCGC»	CCTCCACCA
			CAGIAC	LACATAI	CTACGCGT	GGAGGTGGT
901	TCGTCACAGT AGCAGTGTCA	TTTCTCAGG	TGTAAA	CGCAGTO	GAGGCAAG	ברת ב בידרים
	AGCAGTGTCA	AAAGAGTCCT	YTTKOK	SCGTCAC	CICCGTTC	A DE LA

#### FIGURE 14D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC GTCTAGATCAGATC
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
1351	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
1401	TTATTATGTTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
1451	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGACACCGGGACGTAGGAGACCGGTTGCTACCTAC
	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
1751	CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCCCCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	. TGCAG ACGTC

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## FIGURE 15A



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IGURE 15B

WT preproricin linker

5'- TCGACGGGATATGGTAATGCTGATGTTGT -3' -tctttgcttataaggccastgggggggggaaatttaat--agaaacgaatattçcggicaccacggtttaaaatta-3'- AGCAGTGTCAAAAGACACATAAATGTCCGT

primer VZV-B1

PCR mutagenesis

primer VZV-A2

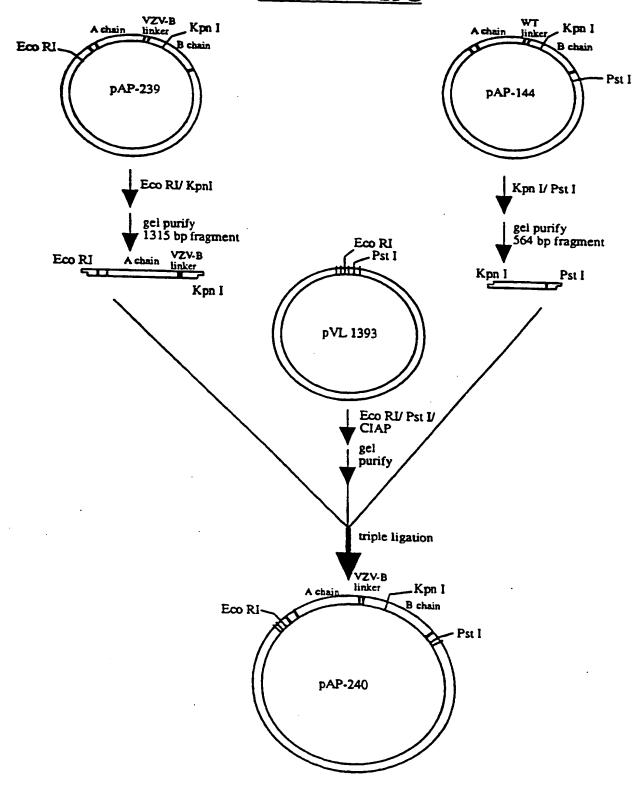
ligate with pBluescript SK

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pAP 239 linker (VZV-B variant) -TCTGTGTATTTACAGGCATCGACGGGATATGGTAAT -AGACACATAAATGTCCGTAGCTGCCCTATACCATTA -----

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#### FIGURE 15C



SUBSTITUTE SHEET (RULE 26)

#### FIGURE 15D

	•	ĭ	20	30		40	•	5
1	GAATTCATG CTTAAGTAC	AAACCGGG: TTTGGCCC	AGGAAA? TCCTTT;	i TTATTA AATADTA	GTAATAI CATTATA	GGATO CCTAC	TATGO	AG'
51	GGCAACATG CCGTTGTAC	GCTTTGTT CGAAACAA	TTGGAT(	CACCTC GTGGAG	AGGGTGG	TCTTT	CACA?	CTA(
101	AGGATAACA TCCTATTGT	ACATATTC( TGTATAAG(	CCCAAA( GGGTTT(	CAATACC STTATGG	CAATTA1 GTTAAT <i>i</i>	raaact Atttgi	TTACC	IAC ITG
151	GCGGGTGCC CGCCCACGG	actgtgca Tgacacgt	AAGCTA( TTCGAT(	CACAAAC GTGTTTG	TTTATCA AAATAG1	AGAGC! PCTCG!	rgttco Acaago	3CG 3GC
201	TCGTTTAAC AGCAAATTG	AACTGGAG TTGACCTC	CTGATG! GACTAC!	IGAGACA ACTCTGT	TGATATA ACTATAT	CCAG:	rgttg(	ICA. IGT
251	ACAGAGTTG TGTCTCAAC	GTTTGCCT. CAAACGGA	ATAAAC( TATTTG(	CAACGGT GTTGCCA	TTATTT AATAAA	PAGTTY	SAACTO CTTGAO	ITC. SAGʻ
301	AATCATGCA TTAGTACGT	GAGCTTTC CTCGAAAG.	TGTTAC: ACAATG:	ATTAGCG TAATCGC	CTGGATO	TCACC	LAATGO	CAT.
351	TGTGGTCGG ACACCAGCC	CTACCGTG GATGGCAC	CTGGAAL GACCTT	ATAGCGC IATCGCG	ATATTTC TATAAAC	TTTC:	TCCT(	BAC.
401	ATCAGGAAG TAGTCCTTC	ATGCAGAA TACGTCTT	GCAATC: CGTTAG:	ACTCATC IGAGTAG	TTTTCAC	TGATO	TTCA AAGTT	LAA'
451	CGATATACA GCTATATGT	TTCGCCTT AAGCGGAA	TGGTGG: ACCACC	TATTAAT ATAATTA	GATAGAC YTOTATO	TTGAL	\CAACI CTTGI	PTG AAC
501	TGGTAATCT ACCATTAGA	GAGAGAAA CTCTCTTT	ATATCG: TATAGC:	AGTTGGG ICAACCC	AAATGG1 TTTACCI	rccac: Aggtgi	PAGAGO	BAG CTC
551	CTATCTCAG GATAGAGTC	CGCTTTAT GCGAAATA	TATTAC: ATAATG'	AGTACTO ICATGAC	GTGGCAC	TCAG(	TTCC/	VAC LTG
601	CTGGCTCGT GACCGAGCA	TCCTTTAT AGGAAATA	AATTTG( TTAAAC(	CATCCAA GTAGGTI	ATGATT:	rcaga. Agtet	AGCAGO ICGTCO	AAS TTE
651	ATTCCAATA TAAGGTTAT	TATTGAGG ATAACTCC	GAGAAA' CTCTTT	TGCGCAC ACGCGTG	GAGAAT? CTCTTAI	raggt: ATCCA!	ACAAC(	:GG. 3CC'
701	GATCTGCAC CTAGACGTG	CAGATCCT GTCTAGGA	AGCGTA TCGCAT	ATTACAC TAATGTO	TTGAGAI	ATAGT PATCA	IGGGG(	BAG. ETC
751	CTTTCCACT GAAAGGTGA	ACTAATTCA TOAATTOO	AGAGTC TCTCAG	TAACCAA ATTGGT1	GGAGCC!	TTTGC:	TAGTCO ATCAGO	CAA STT:
801	TCAACTGCA AGTTGACGT	AAGACGTA TTCTGCAT	ATGGTT TACCAA	CCAAAT1 GGTTTAI	CAGTGTO AGTCACA	STACG:	ATGTG! TACAC!	AGT. ICA
851	TATTAATCO ATAATTAGO	CTATCATA GATAGTAT	GCTCTC CGAGAG	ATGGTG1 TACCAC	TATAGATO ATATCTA	GCGCA CGCGT	CCTCC; GGAGG;	ACC I'GG
901	TCGTCACAC AGCAGTGTC	TTTTTCTGT	GTATTT LCATAAA	ACAGGC!	ATCGACG	GGATA'	TGGTA	ATG

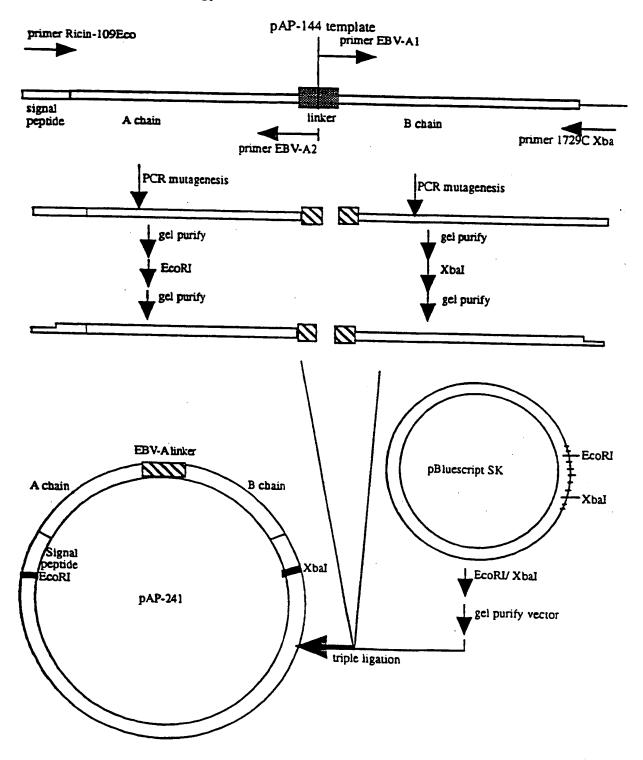
### FIGURE 15D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	ACTICAL CONTROL CONTRO
	ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACAACTACAATCCTACCTACCTACCTACAACGCAATA
	CAGATACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	
1071	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
	THE TENED TO THE TE
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	COMPANY TO THE TAXABLE
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTAGCAATACTGCTGCA
	TAGATACACTAGATACTAACGTTATGACGACGT
1201	A CITICA MODOLA COLOR
1201	ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
	TACCI IGGIAGIATITAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTACA TCACACTACACCACACACACACACACACACACA
	GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
	The state of the s
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
	TOTAL TACGCAAT CAGTT CCAACCGAAGGATGA
1351	A B TTA B TTA CA
	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
	THE COUNTY OF TH
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTI TCA COMPANIA TAGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
3 453	10000000
7427	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
	THE THE PROPERTY OF THE PROPER
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	CTTTMCCGAGATAATIGCCTTACAAGTGATTCTAATATACGGGAAACIGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	- THE TAXABLE CONTROL OF THE TAXABLE CONTROL
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTAC
	THE THE THE TAXABLE CONTROL OF
1601	TCA A CA A MOS MOS A CA A
2001	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
	TOTAL
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
	CACTOCOCTACOCTACACAAATCATTCTTTACCCTCTCCA
	CACTCCGCTAGCCTAGGCTCGGAATTGTTTAGTATAGAAATGGGAGAGGT
	The state of the s
1/01	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTTATACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA
3757	CTCTTCC CTCTCC
	CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	GAGAACGTCACACACAGGACGGTACTTTATCTACCGAATTTATTT
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
	CCTGTAACATTCGAATTCC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATACCTTTAACC

1851 TGCAG ACGTC

#### FIGURE 16A

# PCR Mutagenesis of Preproricin Gene to Create an BBV-A Variant Gene a) Cloning Strategy



TCTAAGCTTGTACAGGCATCGGCGTCAGGTGTTAAT-AGATTCGAACATGTCGTAGCCGCAGTCCACAGTTA-

# IGURE 16B

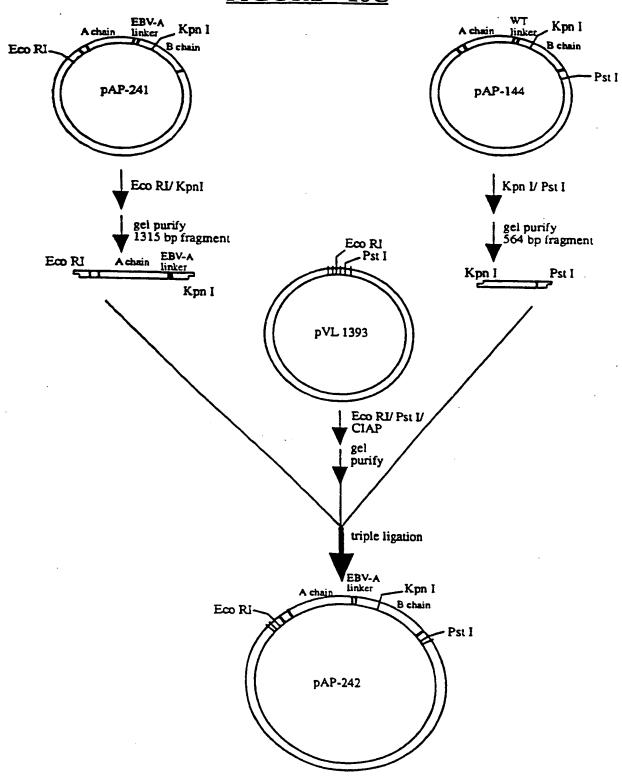
WT preproricin linker

primer EBV-A1

TCTTTGCTTATAAGGCCAGTGCCAAATTTTAAT——TCTTTGCTATATTCCTGTGCTGCCAAATTTTAAATTAAA	primer EBV-A2	PCR mutagenesis	ligate with pBlucscript SK	pAP 241 linker (EB V-A variant)
TC ACAGTGTCAAAA	pri			

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79/254 FIGURE 16C



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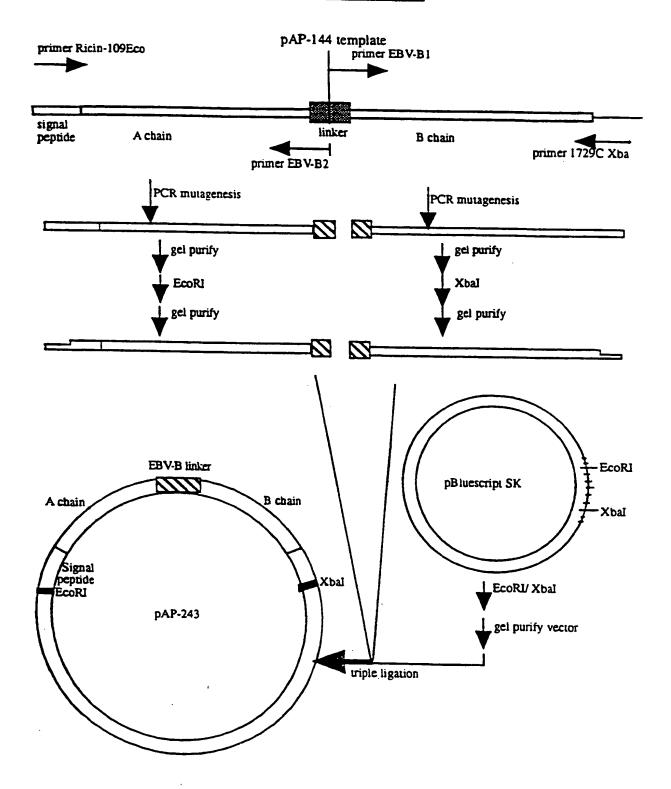
#### FIGURE 16D

		10	20	,	30	2	4	Ó		50
1	GAATTCA: CTTAAGT	TGAAACC	GGGAGG	AAATA	CTATT	GTAA	TATGG	ATGT.	ATGC	 Agt
51	GGCAACA:	IGGCTTT ACCGAAJ	CAAAAC CAAAAC	GATCC CTAGG	ACCT( TGGA(	CAGGG	TGGTC ACCAG	TTTC AAAG	acat Tgta	TAG ATC
101	AGGATAA	CAACATA STTGTAT	TTCCCC	AAACA TTTGT	ATACO	CAAT GTTA	AATAA TTATA	ACTT	TACC	ACA
151	GCGGGTG	CCACTGT	GCAAAG	CTACA	CAAA	لاململىك	moi ex			
	COCCAC	3010MC	icg111c	GATGT	GTTT	SAAAT	'AGTCT	CGAC	AAGC	GCC
201	TCGTTTAI AGCAAAT	ACAACTO IGTTGAC	GAGCTG CTCGAC	ATGTG TACAC	AGACA TCTG1	TADTA ATOA1	'ATACC TATGG	AGTG TCAC	TTGC(	CAA GTT
251	ACAGAGT:	IGGTTTO	CCTATA	AACCA	ACGGT	TATT	TTTAG	TTGA	ACTC	TCA
			-GGWIWI	1-1661	TGCC	AAATA	<b>AAATC</b>	AACT	TGAG	AGT
201	AATCATGO TTAGTACO	CAGAGCT STCTCGA	TTCTGT LAAGACA	TACAT ATGTA	TAGC( ATCG(	CTGG CACC	ATGTC	ACCA TGGT	ATGC:	ATA TAT
351	TGTGGTC	GCTACO	GTGCTG	CALLT	AGCGC	ת מים מי				
	ACACCAG	-CGM1GG	CACGAC	CTTTA	TCGC	STATA	AAGAA	AGTA	GGAC'	IGT
401	ATCAGGA! TAGTCCT	AGATGCA PCTACGT	GAAGCA CTTCGT	ATCAC TAGTG	TCATO AGTAO	TTTT AAAA	CACTG. GTGAC	ATGT TACA	TCAAI AGTT	TAA TTA
451	CGATATA	CATTCGC	CTTTGG	TGGTA	ATTAT	GATA	GACTT	GAAC.	AACT	rcc
501	TGGTAATO									
	ACCATTA	ACTCTC	TTTTAT	AGCTC	AACC	TAAAT	CCAGG	ACTA TGAT	GAGG: CTCC	AGG ICC
551		AGCGCT1	TATTAT	TACAG	TACTO	GTGG	CACTO	AGCT	TCCAI	ACT
501		COCON	WINWIN	MIGIC	ATGAC	CACC	GTGAG	TCGA	AGGT:	TGA
	CTGGCTC		ALT ALT TA	MACGI	AGGT	TACT	AAAGT	CITC	GTCG:	TTC
651	ATTCCAA! TAAGGTT	OKKTATA SAATATA	AGGGAG TCCCTC	AAATG TTTAC	CGCAC	GAGA CTCT	ATTAG	GTAC.	AACC( TTGG(	GGA CCT
701		ACCAGAT	CCTAGO	יי ע עייטי	יאר א מידי	-mm- *	<b>~~~</b>			
751	CTTTCCA( GAAAGGT	TGCAA1	TCAAGA	СТСТА	ACCA I				_	
801										
	TCAACTG AGTTGAC	STTTCTO	CATTAC	CAAGG	TTTAL	ICAGI AGTCA	GTGTA CACAT	CGAT GCTA	GTGA( CACT(	GTA Cat
851	TATTAAT	CCTATO	ATAGCT	CTCAT	GGTG	PATAG	ATGCG	CACC	TCCA	CA
			31VICON	IGWG I W	CCAC	TATC	TACGC	GTGG.	aggty	<b>G</b> T
201	TCGTCAC:	TCAAAA	ATTCGA	ACATG	AGGC! TCCG:	ATCGG IAGCC	CGTCA CCAGT	GGTG CCAC	TTAA! AATT!	rgc Acg

# FIGURE 16D (CONT'D)

951	${\tt TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATGACTACCAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC$
1001	${\tt GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT}$
1051	${\tt CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA}$
1101	${\tt GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACGCTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC}\\$
1151	${\tt GGTACAGTCCGGGAGTCTATGTGATGATTGTATGCAATACTGCTGCACATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT}$
1201	lem:lem:lem:lem:lem:lem:lem:lem:lem:lem:
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACACGTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG ACGTC

#### FIGURE 17A



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FIGURE 17B

WT preproricin linker

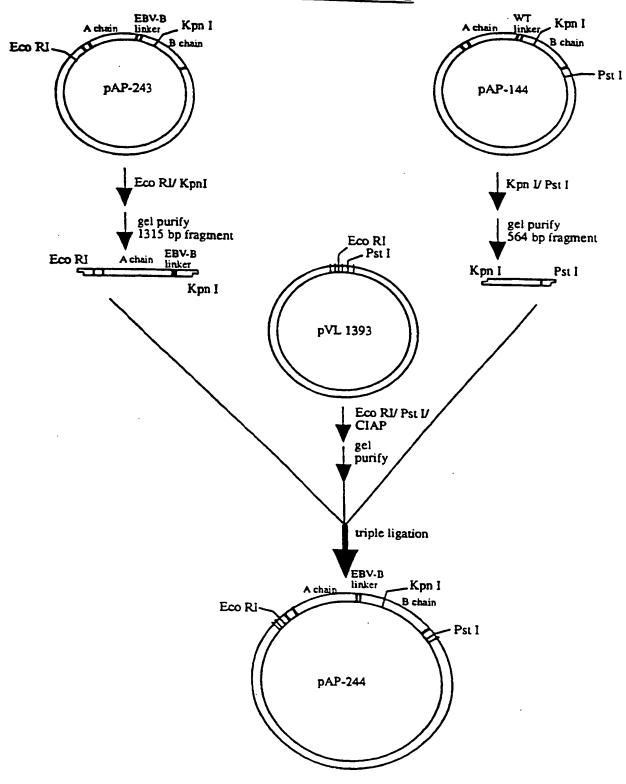
primer EBV-B1

5'- TCGGACGCACCTGATAATGCTGATGTTTGT ligate with pBluescript SK - TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT---AGAAĄCGĄAŢATTÇCGGT[CACCACGGTTTAAAATTA-PCR mutagenesis 3'- AGCAGTGTCAAAAGAAGCATAGATTTCCGT-5' primer EBV-B2

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pAP 243 linker (EBV-B variant) 

# FIGURE 17C



SUBSTITUTE SHEET (RULE 26)

# FIGURE 17D

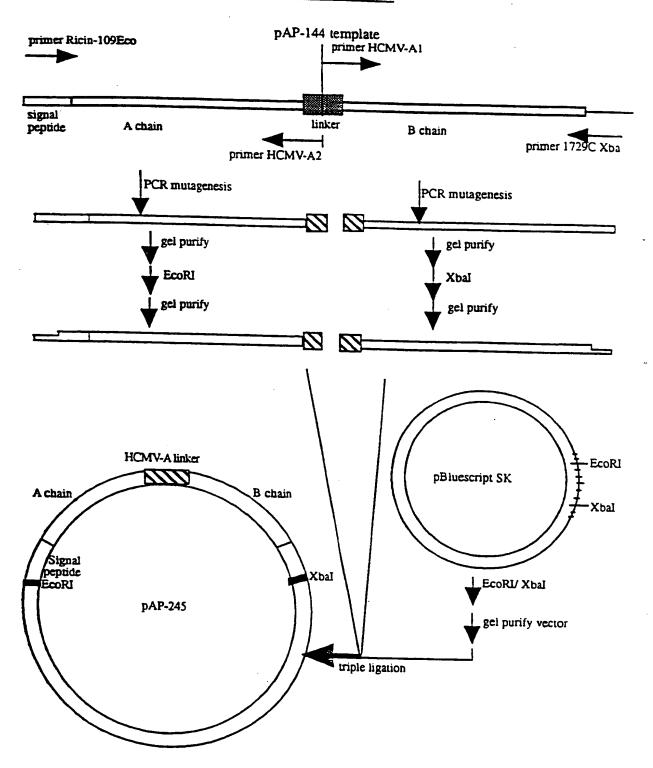
	10	20	30	40	50
1	GAATTCATGAAACC CTTAAGTACTTTGG	GGGAGGAAA' CCCTCCTTT	IACTATTGTAA ATGATAACATT	 TATGGATGTA ATACCTACAT	TGCAGT ACGTCA
51	GGCAACATGGCTTT	GTTTTGGAT	CCACCTCAGGG	TGGTCTTTCA	CATTAG
	CCGTTGTACCGAAA	CAAAACCTA	GGTGGAGTCCC	ACCAGAAAG1	CTAATC
101	AGGATAACAACATA	TTCCCCAAA	CAATACCCAAT	TATAAACTT	TACCACA
	TCCTATTGTTGTAT	AAGGGGTTT	GTTATGGGTTA	ATATTTGAAJ	ATGGTGT
151	GCGGGTGCCACTG1	GCAAAGCTA	CACAAACTTTA	TCAGAGCTG:	TTCGCGG
	CGCCCACGGTGACA	CGTTTCGAT	GTGTTTGAAAT	AGTCTCGAC	VAGCGCC
201	TCGTTTAACAACTC	GAGCTGATG	TGAGACATGAT	ATACCAGTG:	TTGCCAA
	AGCAAATTGTTGAC	CTCGACTAC	ACTCTGTACTA	TATGGTCAC	AACGGTT
251	ACAGAGTTGGTTTG	CCTATAAAC	CAACGGTTTAT	TTTAGTTGA	ACTCTCA
	TGTCTCAACCAAAC	GGATATTTG	GTTGCCAAATA	AAATCAACT	FGAGAGT
301	AATCATGCAGAGCT	TTCTGTTAC	ATTAGCGCTGG	ATGTCACCAL	ATGCATA
	TTAGTACGTCTCGA	AAGACAATG	TAATCGCGACC	TACAGTGGT	PACGTAT
351	TGTGGTCGGCTACO	GTGCTGGAA	ATAGCGCATAT	TTCTTTCAT(	CCTGACA
	ACACCAGCCGATGO	GCACGACCTT	TATCGCGTATA	AAGAAAGTA	GGACTGT
401	ATCAGGAAGATGC: TAGTCCTTCTACGT	AGAAGCAATC TCTTCGTTAG	ACTCATCTTT TGAGTAGAAA	CACTGATGT:	CAAAAT AGTTTTA
451	CGATATACATTCGC	CTTTGGTGG	TAATTATGATA	GACTTGAACI	AACTTGC
	GCTATATGTAAGCC	GAAACCACC	ATTAATACTAT	CTGAACTTG	ITGAACG
501	TGGTAATCTGAGAC	SAAAATATCG	AGTTGGGAAAT	GGTCCACTA	SAGGAGG
	ACCATTAGACTCTC	CTTTTATAGC	TCAACCTTTA	CCAGGTGAT	CTCCTCC
551	CTATCTCAGCGCTT	TATTATTAC	AGTACTGGTGC	CACTCAGCT	TCCAACT
	GATAGAGTCGCGAI	ATAATAATA	TCATGACCACC	CGTGAGTCGA	AGGTTGA
601	CTGGCTCGTTCCTT	TTATAATTTG	CATCCAAATGI	ATTTCAGAAG	CAGCAAG
	GACCGAGCAAGGA	AATATTAAAC	GTAGGTTTACT	OTTCTDAAAA	GTCGTTC
651	ATTCCAATATATTC	Gagggagaaa	TGCGCACGAGI	ATTAGGTAC.	AACCGGA
	TAAGGTTATATAA	CTCCCTCTTT	ACGCGTGCTC	TTAATCCATG	TTGGCCT
701	GATCTGCACCAGA	TCCTAGCGTA	ATTACACTTGA	AGAATAGTTG	GGGGAGA
	CTAGACGTGGTCT	AGGATCGCAT	TAATGTGAACT	CAACTATTCA	CCCCTCT
751	CTTTCCACTGCAA	TTCAAGAGT(	TAACCAAGGA(	GCCTTTGCTA	GTCCAAT
	GAAAGGTGACGTT	AAGTTCTCA(	SATTGGTTCCT(	CGGAAACGAT	CAGGTTA
801	TCAACTGCAAAGA	CGTAATGGTT	CCAAATTCAG	TGTGTACGAT	GTGAGTA
	AGTTGACGTTTCT	GCATTACCAJ	GTTTAAGTC	ACACATGCTA	CACTCAT
851	TATTAATCCCTAT	CATAGCTCT(	CATGGTGTATA	GATGCGCACC	TCCACCA
	ATAATTAGGGATA	GTATCGAGA(	STACCACATAT	CTACGCGTGG	AGGTGGT
901	TCGTCACAGTTTT AGCAGTGTCAAAA	CTTCGTATC:	TAAAGGCATCG ATTTCCGTAGC	GACGCACCTG CTGCGTGGAC	ATAATGC TATTACG

# FIGURE 17D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	THE TRUE TAGGAC TEGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACACACACACACACACACACACACACACACACACA
	CAGATACACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	THE TOTAL PARTICULAR PROPERTY OF THE PROPERTY
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTAGCATACGACGTCA
	TACGTTATGACGACGT
1201	ACTGATGCCACCGGTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGCGACCCTTTATAGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTAGATCAGAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTGTG
1201	THE CONTROL OF THE CO
T2 0 T	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
	The state of the s
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
	THE THE THE THE TANK
1401	CTTGCAAGCAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
	THE CONTROL OF THE PROPERTY OF
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
	TOTAL CARGITATGCAGGAGTC
1201	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	THE TAIL THE THE TAIL THE TAIL THE THE TAIL THE THE THE THE THE THE THE THE
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGACACCGGGACGTAGGACGATGCTACAAACCAACGATGCTACAAACAA
	THE
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
	THE CALCUTA CCACACATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
	CACTCCGCTAGCCTAGGCTCGGAATTTCTTTACCCTCTCCA
	CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTATAGAAATTGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTTATACCALTGGTTATTTTGATAGACAGATTACT
	TATACCAA IGG TAA TAAAACTATCTGTCTAA TGA
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	GAGAACGTCACACACACACGCCACGCCATGAAAATAGATGGCTTAAATAAA
	TO THE TOUR COURSE OF THE TATE
1801	GGACATTGTA A ATTTTCTA A CMCA A
	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG
	ACGTC
	2004

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# FIGURE 18A



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# IGURE 18B

# WT preproricin linker

primer HCMV-A1

5'- TCGTGTAGACTTGCTAATGCTGATGTTGT -3' ligate with pBluescript SK -tctttgcttataaggccastgggggggggggtaaaatttaat--agaaagagaatattççggtgacacggtttaaaatta-PCR mutagenesis 3'- AGCAGTGTCAAAAGACCCCAACATTTACGT-5' primer HCMV-A2

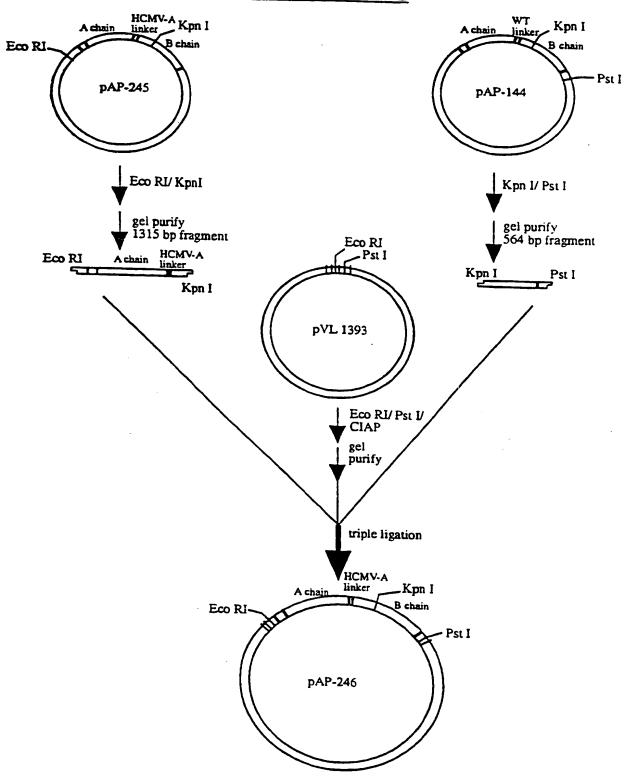
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pAP 245 linker (HCMV-A variant)

-TCTGGGGTTGTAAATGCATCGTGTAGACTTGCTAAT--AGACCCCAACATTTACGTAGCACATCTGAACGATTA-

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#### FIGURE 18C



SUBSTITUTE SHEET (RULE 26)

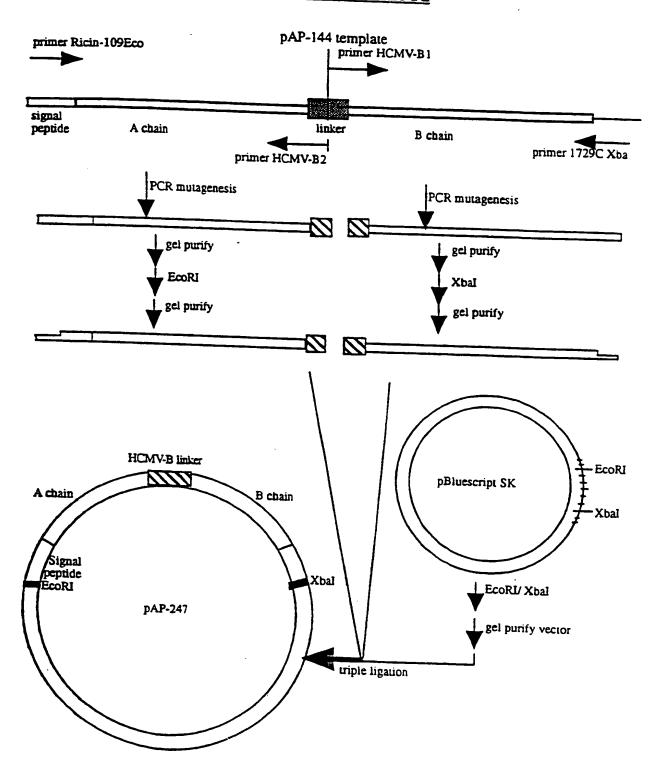
# FIGURE 18D

	10	20	30	40	50
1	GAATTCATGAAACC CTTAAGTACTTTGG	GGGAGGAAA' CCCTCCTTT	TACTATTGTAA ATGATAACATT	 TATGGATGT: ATACCTACA	 ATGCAGT ACGTCA
51	GGCAACATGGCTTT CCGTTGTACCGAAA	יה ג בובות התבב			
101	AGGATAACAACATA TCCTATTGTTGTAT	דייייי איי	C		
151	GCGGGTGCCACTGT CGCCCACGGTGACA	GCAAAGCTA	C) C) ) )		
201	TCGTTTAACAACTG AGCAAATTGTTGAC	GAGCTGATG	TC		
251	ACAGAGTTGGTTTG TGTCTCAACCAAAC	מ מ מיי מיים	C 3 3 CCC		
301	AATCATGCAGAGCT	ے لابلیٹ کیلیکیلی <u>۔</u>	7 TT 7 CCCC		
351	TGTGGTCGGCTACC	GTGCTCGA N	AATCGCGACC	TACAGTGGT	TACGTAT
	ATCAGGAAGATGCA	GAAGCAATC	ATCGCGTATA	AAGAAAGTA	GGACTGT
	CGATATACATTCGC	CTTTCCTCC	TA A TITA TON ON	GTGACTACA	AGTTTTA
501			ATTAATACTAT	CTGAACTTG	TTGAACG
	TGGTAATCTGAGAG. ACCATTAGACTCTC		I CAMCCCTTTA	CCAGGTGAT	CTCCTCC
551	GATAGAGTCGCGAA		TCHIGHCCACC	GTGAGTCGA	AGGTTGA
601	CTGGCTCGTTCCTT GACCGAGCAAGGAA		C) = C = -		
651	ATTCCAATATATTG TAAGGTTATATAAC	AGGGAGAAA	MCCCCC		
701	GATCTGCACCAGAT CTAGACGTGGTCTA	CCTA GCCTA	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \		
751			M > 2 - 2 - 2		
801	TCAACTGCAAAGAC AGTTGACGTTTCTG	GTA A TGCTT	CC)		
	TATTAATCCCTATC	ATACCTCTC	ogiliaagica	CACATGCTA	CACTCAT
	TCGTCACAGTTTTC	Teccemen	ACCACATATC	TACGCGTGG	AGGTGGT
	AGCAGTGTCAAAAG	ACCCCAACA	TTTACGTAGCA	CATCTGAAC	GATTACC

#### FIGURE 18D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCCAGAGAGAGAGAGAGA
	TTACAGTGCAAACCAACATTTTATGCCCTTTACACCATGGTGTG
	AATAATACACAACCTTTTGTTACAACGAATCAGTTCCAACCGAAGGATGA
	TO THE TOTAL PARTY OF THE TAX CARCACTE GATATACCAGACAC
	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
	${\tt AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAGTCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTCCGAGGAGTCACCAAGTTATGCAGGAGTCCGAGGAGTCACCAAGTTATGCAGGAGTCCGAGGAGTCACCAAGTTATGCAGGAGTCCAAGTTATGCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGGAGTCAAGTTATGCAGAGATGCAGAGTCAAGTTATGCAGAGATGCAAGTTATGCAGAGATGCAGAGATGCAAGATGAAGATGCAAGATGAAGATGAAGATGAAGATGAAGATGAAGATGAAGATGAAGATGAAGAA$
	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCCCACACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGAGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG ACGTC

# 92/254 FIGURE 19A



IGURE 19B

WT preproricin linker

primer HCMV-B1

3 - AGCAGTGTCAAAAGAAGCATACATTTCCGT-5'

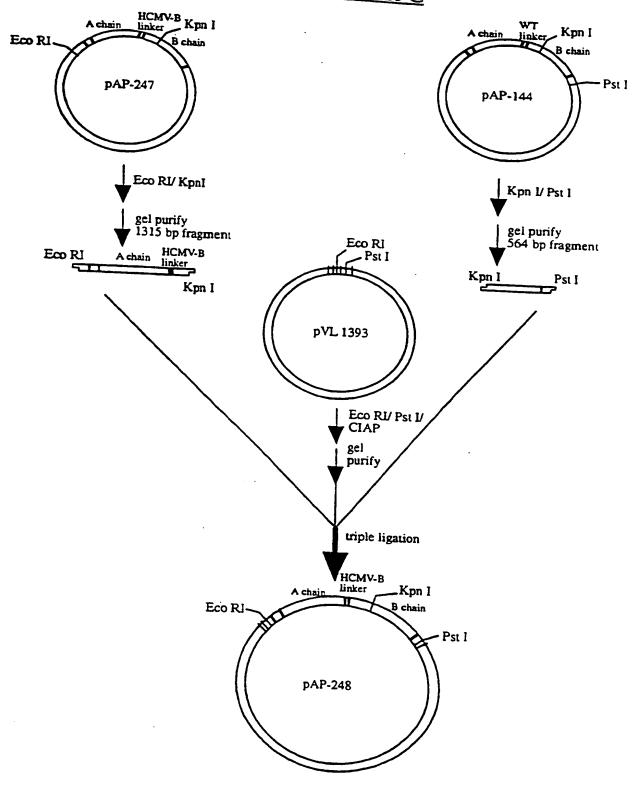
primer HCMV-B2

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PCR mutagenesis
Ilgate with pBluescript SK

pAP 247 linker (HCMV-B variant) -TCTTCGTATGTAAAGGCATCGGTGTCACCTGAAAAT—-AGAAGCATACATTTCCGTAGCCACAGTGGACTTTTA—

# FIGURE 19C



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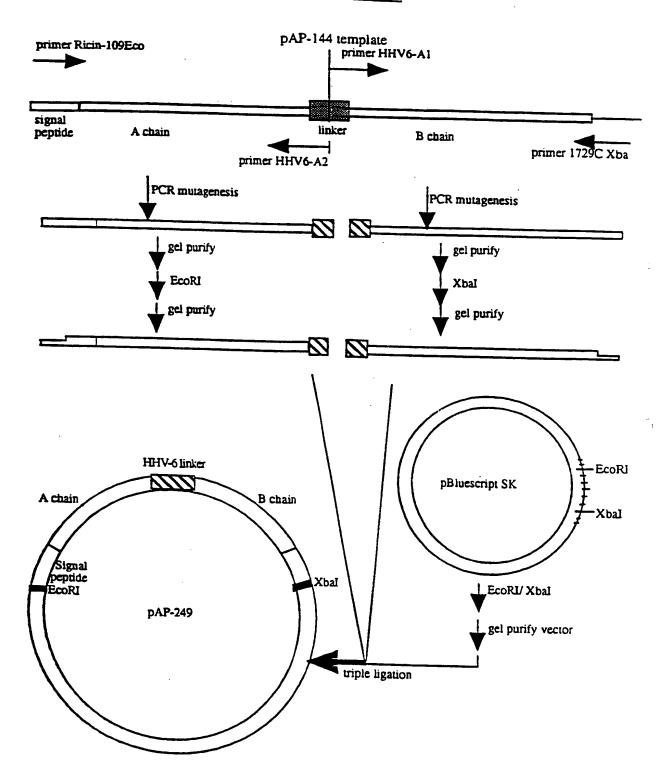
# FIGURE 19D

	•				
	10	20 I	30	40	50
1	GAATTCATGAA CTTAAGTACTT	accgggagģaaa Iggccctcctti	AATƏTTATƏAT. TACAATAƏTA	TATGGÅTGTA TATACCTACAT	ITGCAGT PACGTCA
51	GGCAACATGGC CCGTTGTACCG	TTTGTTTTGGAT AAACAAAACCTA	CCACCTCAGGC GGTGGAGTCC	TGGTCTTTC	CATTAG CTAATC
101	AGGATAACAAC TCCTATTGTTG	ATATTCCCAAA TATAAGGGGTTT	CAATACCCAA?	TTATAAACTT AATATTTGAA	FACCACA ATGGTGT
151	GCGGGTGCCAC CGCCCACGGTG	TGTGCAAAGCTI ACACGTTTCGA1	CACAAACTTT GTGTTTGAAA	ATCAGAGCTG' TAGTCTCGAC	TTCGCGG AAGCGCC
201	TCGTTTAACAA AGCAAATTGTT	CTGGAGCTGATO CACCTCGACTA	STGAGACATGA CACTCTGTACT	TATACCAGTG ATATGGTCAC	TTGCCAA AACGGTT
251	ACAGAGTTGGT TGTCTCAACCA	TTGCCTATAAA AACGGATATTT	CCAACGGTTTA GGTTGCCAAAT	TTTTAGTTGA AAAATCAACT	ACTCTCA TGAGAGT
301	AATCATGCAGA TTAGTACGTCT	ACCTTTCTGTTA CGAAAGACAAT	CATTAGCGCTG GTAATCGCGAC	GATGTCACCA CTACAGTGGT	ATGCATA TACGTAT
351	TGTGGTCGGCT	raccgtgctgga Atggcacgacct	AATAGCGCATA TTATCGCGTAT	TTTCTTTCAT AAAGAAAGTA	CCTGACA GGACTGT
401	ATCAGGAAGA: TAGTCCTTCT	TGCAGAAGCAAT ATTDOTTOTOA	CACTCATCTTT GTGAGTAGAAA	TCACTGATGT AGTGACTACA	TCAAAAT AGTTTTA
451	CGATATACAT	ICGCCTTTGGTG AGCGGAAACCAC	GTAATTATGAT CATTAATACTA	'AGACTTGAAC TCTGAACTTG	AACTTGC
501	TGGTAATCTG.	AGAGAAAATATC PCTCTTTTATAG	GAGTTGGGAAA CTCAACCTTT	TGGTCCACTA CACCAGGTGAT	GAGGAGG CTCCTCC
551	CTATCTCAGC GATAGAGTCG	GCTTTATTATTA CGAAATAATAA	CAGTACTGGTC	GCACTCAGCT CGTGAGTCGA	TCCAACT AGGTTGA
603	CTGGCTCGTT GACCGAGCAA	CCTTTATAATTI GGAAATATTAA	GCATCCAAATO ATTTDDATGTTA	SATTTCAGAAC CTAAAGTCTTC	CAGCAAG CGTCGTTC
65:	ATTCCAATAT TAAGGTTATA	ATTGAGGGAGAI TAACTCCCTCT	ATGCGCACGAC TTACGCGTGCTC	GAATTAGGTAG CTTAATCCATG	LAACCGGA STTGGCCT
70:	GATCTGCACO CTAGACGTGG	AGATCCTAGCG: TCTAGGATCGC	TAATTACACTTY ATTAATGTGAA	GAGAATAGTT( CTCTTATCAA	GGGGAGA CCCCTCT
75	1 CTTTCCACTO GAAAGGTGAO	CAATTCAAGAG CTTAAGTTCCC			
80	1 TCAACTGCAA AGTTGACGTT	AGACGTAATGG TCTGCATTACC	TTCCAAATTCA AAGGTTTAAGT	GTGTGTACGA CACACATGCT	TGTGAGTA ACACTCAT
85	1 TATTAATCC	CTATCATAGCTC GATAGTATCGAG	TCATGGTGTAT AGTACCACATA	AGATGCGCAC TCTACGCGTG	CTCCACCA GAGGTGGT
90	1 TCGTCACAG	ITTTCTTCGTAT	CTAAAGGCATO OATTTTCCAA	GGTGTCACCT	GAAAATGC

# FIGURE 19D (CONT'D)

951	TGATGTTTCTATCCATCCATC
	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAARAGAGACAATA CTRATTOGA COLOR CARACTERISTICA CARAC
	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGG1GTCT1TCTC1TG1TG1TG1TG1TG1TG1TG1TG1TG1TG1TG1TG1TG1
	TACTACTACTACTAACGTTATGACGACGT
	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
	THE TOTAL STREET OF THE TOTAL STREET, THE TOTAL
1301	TTACAGTGCAAACCAACA TTTTA TTCCCTTTT
	THE THE CONTROL OF THE PROPERTY
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTCCTAACAACCATTGTTGGGCTATATGGTCTGTG
	THE
1401	CTTGCAAGCAATAGTGGACAAGTAGT
	TOTAL TOTAL CONTROL OF THE PROPERTY OF THE PRO
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CANALOGO
1301	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAACAGT
	TOTAL TANGET AT THE TOTAL AND THE TANGET AND THE TA
1111	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	THE THE CONTROL OF THE CANADA
TOOT	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACTGTATAGTGGTTTAGAT
	THE TABLE TABLE TO A TOTAL TOT
1651	GTGAGGCGATCGGATCCGACCCTTTANA
	THE TAKE THE
1701	TGGTGACCCAAACCAAACCAAATTATTCCTTTA COORDA
	TO THE PROPERTY OF THE PROPERTY AND A TICK
1751	CTCTTGCAGTGTGTGTCTCCTCCC
	The Later of the L
1801	GGACATTGTAAATTTTCTAACCCAACCCAACCCAACCCA
	THE THE TENT OF TH
TR21	TGCAG ACGTC

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# FIGURE 20F

# WT preproricin linker

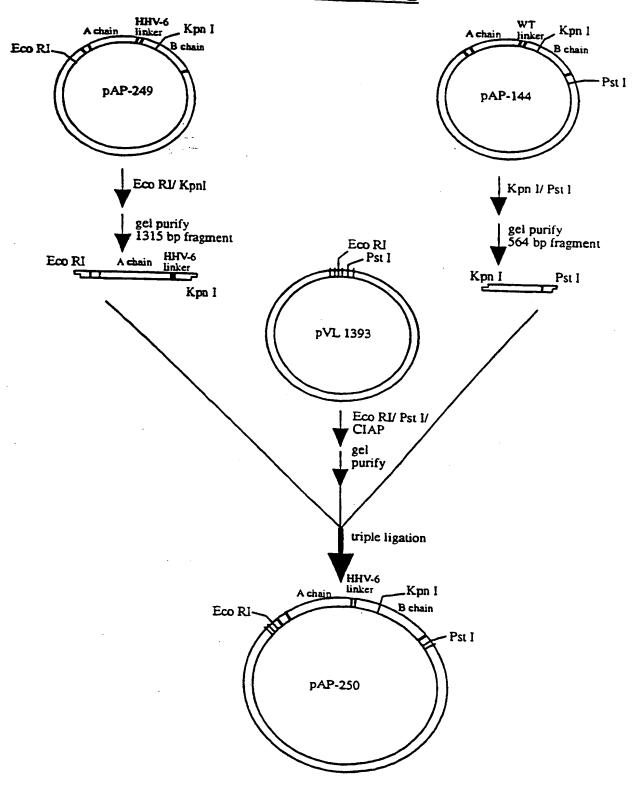
primer HHV6-A1

5'- TCGGTGCCAAATTTTAAT GTGGTGCCAAATTTTAAT-CACCACGGTTTAAAATTA ligate with pBluescript SK PCR mutagenesis 3'- AGCAGTGTCAAAAGAAGCTAAAATTTACGT-5 primer HHV6-A2

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pAP 249 linker (HHV-6 variant) --- TCTTCGATTTTAAATGCATCGGTGCCAAATTTTAAT --- AGAAGCTAAAATTTACGTAGCCACGGTTTAAAATTA

# FIGURE 20C



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# FIGURE 20D

	10	20	30		
,	G3330000	- 7		40	50
د	GAATTCATGAA CTTAAGTACTT	ACCGGGAGGAA	ATACTATTGTAZ	TATGGATCHA	<b>7</b> 222 - 1
			OVINVCVI	ATACCTACAT	ACCTCa
51	. GGCAACATGGC	المحاصية المحاسبة المحاسبة			
	CCGTTGTACCG	AAACAAAACCT	TCCACCTCAGGG AGGTGGAGTCCC	TGGTCTTTCA	CATTAG
				-ACCAGAAACT	こて ひ カ かへ
101	. AGGATAACAAC	ATATTCCCC	• • • • • •		
	TCCTATTGTTG	TATAAGGGGTT	ACAATACCCAAT IGTTATGGGTTA	TATAAACTTT	ACCACA
151	C0000000			LATATTTGAAA	iggigi
*	GCGGGTGCCAC CGCCCACGGTG	TGTGCAAAGCT	ACACAAACTTTE	TC 3 C 3 C C C C C C C C C C C C C C C C	
	CGCCCACGGTG	ACACGTTTCGA'	TGTGTTTGAAAT	ACTUTUUT OF C	TCGCGG
201	ער אינייידים איני	OB000			AGCGCC
	TCGTTTAACAA AGCAAATTGTT	CIGGAGCTGAT	GTGAGACATGAT	'ATACCAGTGT	TCCCAA
				TATEGTORE	
251	ACAGAGTTGGT TGTCTCAACCA	TTCCCT2 =			100011
	TGTCTCAACCA	TOCCIMINAN(	CAACGGTTTAT	TTTAGTTGAA	こすてすてな
				AAATCA I CTTT	23 C 3 C M
301	AATCATGCAGA				
	TTAGTACGTCT	CGAAAGACAATY	-ATTAGCGCTGG	ATGTCACCAA:	<b>IGCATA</b>
			COCOMCC	TACACTCCTT	2 CCM2 M
351	TGTGGTCGGCT:				
	ACACCAGCCGA	IGGCACGACCT	TATCCCCTATAT	TTCTTTCATC	TGACA
			COCGININ	AAGAAAGTACC	2 A C T C T C T
401	ATCAGGAAGATO TAGTCCTTCTAG	CAGAAGCAATC	ACTCATCTTTT	C	
	TAGTCCTTCTAG	CGTCTTCGTTAC	TGAGTAGAAA	CACTGATGTTC	TAAAA
451	CGATATACA			GIGACTACAAC	TTTTA
	CGATATACATTO GCTATATGTAAO	GCCTTTGGTGG	TAATTATGATA	GACTTGAACAA	CORROR
				C.I.CY V CLALAC	2022
501	IGGTAATCTCA				
	ACCATTAGACTO	MCAMMAN WA CO	AGTTGGGAAAT	GGTCCACTAGA	GGAGG
				ニヒみにいてひかつす	·~~~~
551	CIMICICACCC				
	GATAGAGTCGC	AAATAATAATC	AGTACTGGTGG(	CACTCAGCTTC	CAACT
		-		- 11 - 41 - 11 - 12 - 13 - 14	
<b>601</b>	CIGGLICGITC	- The State of the Party of the State of the			
	GACCGAGCAAGG	AAATATTAAAC	GTACCTTTACT	LTTCAGAAGCA	GCAAG
		-		AAAL II TIITOO	
031	ATTCCAATATATATATATATATATATATA	TGAGGGAGAAA	TGCGCACGAGA	Trm2 CCm2 c2 -	
	TAAGGTTATATA	ACTCCCTCTTT	ACGCGTGCTCTT	LY Y LCC Y WCWW Y T Y GG LWCWY	CCGGA
701	GATCTGCACCAC	1 man-			GGCCT
	GATCTGCACCAG CTAGACGTGGTC	ATCCTAGCGTA	ATTACACTTGAC	AATAGTTGGG	CCNCN
751	CITTCCACTCCA	D 1000 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			
	GAAAGGTGACGT	TAAGTTCTCA.	TAACCAAGGAGG	CTTTGCTAGT	CCAAT
801	- JUANCIGE A A A C	ACCOUNT MOSSON			
	AGTTGACGTTTC	TGCATTACCAA	CCAAATTCAGTC	TGTACGATGT	GAGTA
801	TATTAATCCCT1	TC 2 T2 COMO			
	ATAATTAGGGAT	AGTATCGAGAG	TACCACATATACA	LIGCGCACCTC	CACCA
201	TCGTCACAGTTT AGCAGTGTCAAA	TCTTCGATTTT	AAATGCATCGGT	CCC 2 2 2	
	AGCAGTGTCAAA	AGAAGCTAAAA:	TTTACGTAGCCA	CCCAMMY * * * *	AATGC
	1			-GGTTTWAAA	LLYCC

# FIGURE 20D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATGACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	
	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATG
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACACGTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCCTTACTGAACGAAC
	AATAATACACAACCTTTTGTTACAACGCAATCAGTTCCAACCGAAGGATGA
	THE TOTAL CARLES TO THE TENT OF THE TENT O
	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAGTCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG ACGTC

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#### FIGURE 21

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-213/pAP-214 linker (Cathepsin B):

A chain- S L L K S R M V P N F N -B chain

pAP-215/pAP-216 linker (MMP-3):

A chain- R P K P Q Q F F G L M N -B chain

pAP-217/pAP-218 linker (MMP-7):

A chain- S L R P L A L W R S F N -B chain

pAP-219/pAP-220 linker (MMP-9):

A chain- S P Q G I A G Q R N F N -B chain

pAP-221/pAP-222 linker (THERMOLYSIN-LIKE MMP):

A chain- D V D E R D V R G F A S F L -B chain

pAP-241/pAP-242 linker (EBV-A):

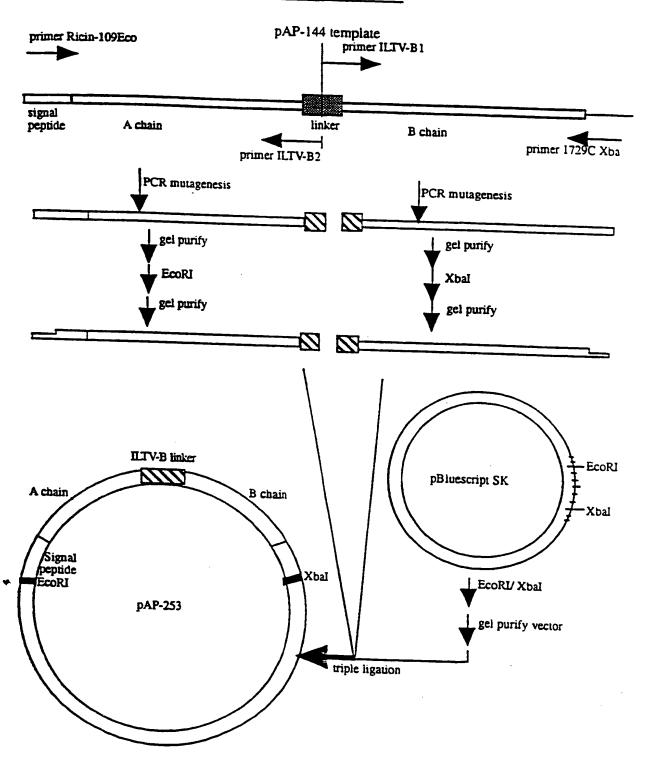
A chain- S K L V Q A S A S G V N -B chain

pAP-243/pAP-244 linker (EBV-B):

A chain- S S Y L K A S D A P D N -B chain

#### SUBSTITUTE SHEET (RULE 26)

#### FIGURE 22A



#### SUBSTITUTE SHEET (RULE 26)

# IGURE 22B

WT preproricin linker

primer ILTV-B1	5'- AATGAGGTAATTACTAATGCTGATGTTTGT -3'	TCTTTGCTTATAAGGCCAGTGCCAAATTTTAAT—AGAĄĄCGĄĄAĮTTÇCGGTCACCAGGTTTAAAATTA	1-5,
prim	-,5	TCTTTGCTTATAAGGCC	3'- AGCAGTGTCAAAAGATTCATAGATGTCCGT-5'

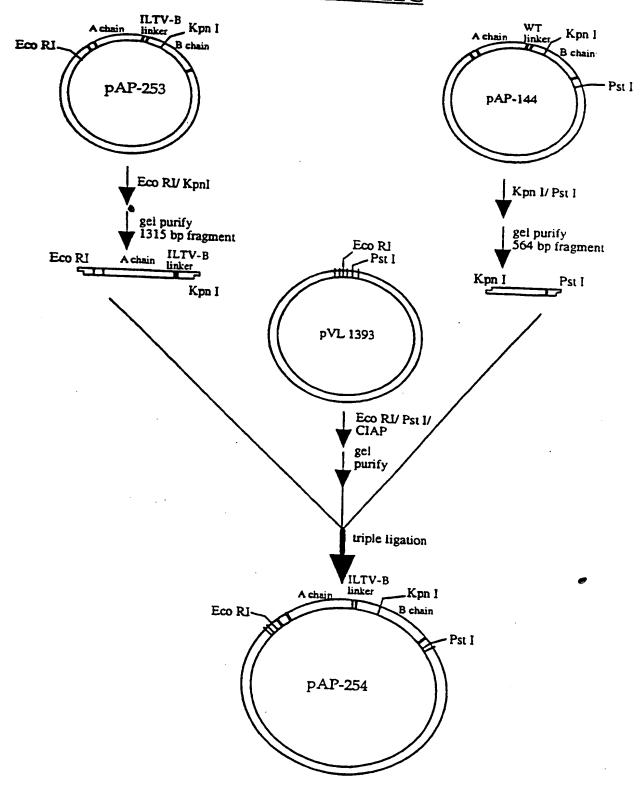
primer ILTV-B2

PCR mutagenesis ligate with pBluescript SK

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pAP 253 linker (ILTV-B variant) --- TCTAAGTATCTACAGGCAAATGAGGTAATTACTAAT ---- AGATTCATAGATGTCCGTTTACTCCATTAATGATTA ----

# FIGURE 22C



SUBSTITUTE SHEET (RULE 26)

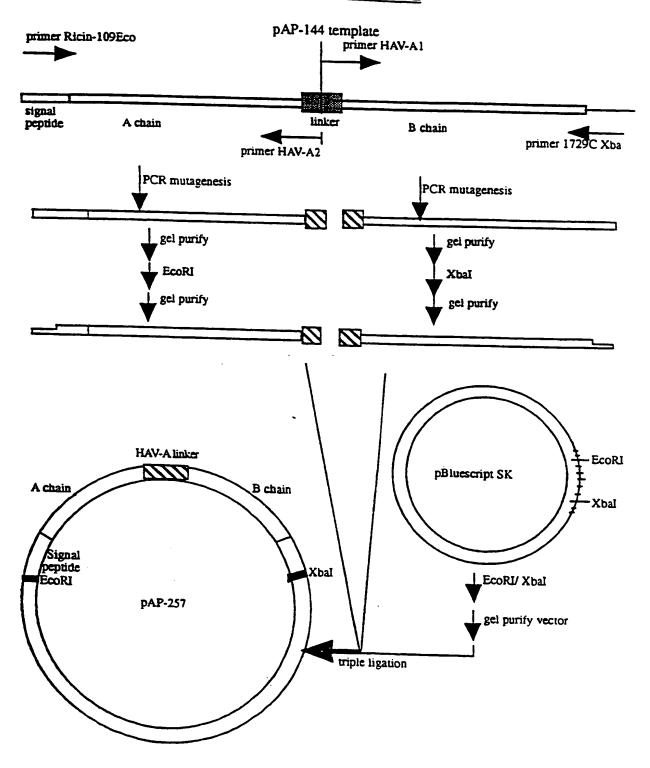
# FIGURE 22D

		10	20	. 30	40	
1	GAATTCA	IGAAACCGG	GACCAAA		40    ATATGGATG	50 1
				WIGNINACA!	TATACCTAC	ATACGTCA
51	GGCAACA'	rggctttgt	TTTGGAT	CACCTCACC	GTGGTCTTI	
			:	ag regarct.C.C	CACCAGAAA	GTGTAATC
101	AGGATAA	CAACATATT	*******	~		
				at TWI GGGL.I	:AATATTTGA	AATGGTGT
151	GCGGGTG	CACTGTGC	AAAGCTA	-ACAAACTTT	ATCAGAGCT	
				31GTTTGAAA	TAGTCTCGA	CAAGCGCC
201	TCGTTTA	ACAACTGGa	CCTCNOC	803.03.00		
			- LINCINC	ACTUIGINCY	ATATGGTCA	CAACGGTT
251	ACAGAGT	rggtttgcc	TATAAAC	AACGGTTT n	TTTTAGTTG	
	TGTCTCAL	ACCAAACGG	ATATTTG	TTGCCAAAT	TTTTAGTTG `AAAATCAAC	AACTCTCA TTGAGAGT
301	AATCATG	AGAGCTTT	~~~~~~~~~~			
	TTAGTAC	STCTCGAAA	CIGITACA	VITAGCGCTG	GATGTCACC CTACAGTGG	AATGCATA
				WATCACAK	CTACAGTGG	TTACGTAT
351	ACACCACC	SGCTACCGT	GCTGGAA	TAGCGCATA	TTTCTTTCA	TECTEN
				WI COCO IMI	aaagaaagt	AGGACTGT
401	ATCAGGAZ	GATGCAGA	2002200			
	TAGTCCTT	CTACGTCT	TCGTTAGT	GAGTAGAAA	TCACTGATG AGTGACTAC	TTCAAAAT
451	CGATATAC	~				AAGT-T-T-TA
	GCTATATO	ATTUGUET TAAGUGG	TTGGTGGT	AATTATGAT	AGACTTGAA	CAACTTGC
				TINKINCIA	TCTGAACTT	GTTGAACC
501	TGGTAATC	TGAGAGAA	AATATCGA	GTTGGGAAA	TGGTCCACT.	30300
	ACCATTAC	ACTCTCTT	TTATAGCT	CAACCCTTT	TGGTCCACT. ACCAGGTGA	AGAGGAGG TCTCCTCC
551	CTATCTCZ	الاستساعات	TT 3 TT			
				CATOACCAC	GCACTCAGC CGTGAGTCG	AAGGTTC A
601	CIGGCIC	و لا سلسل السائد	The same of			
				TAGGILIAC	TAAAGTCTT	<u>್ರಾಗ್ ಬಿಸ್ಟಾರ್</u>
651	ATTCCAAT	ATATTGAC	GGAGAAA	V0000000		
	9			reace tection	TTAATCCAT(	STYGGCCT
701	GATCTGC	CCAGATCC'	TAGCGTAA	TTACACTTC	AGAATAGTT	
				TO TOWAL.	<b>ICITATCAA</b> (	
751	CTTTCCAC	TGCA A TTC	× × × × × × × × ×			
					-GGAAACGA?	じこみにこかかっ
801	TCAACTGC	AAAGACGT	7 7 77 77 7 7			
				GI I I I WG I C	AUACATGCTI	とし として とり
851	TATTAATC	CCTATCATO	* CCMC===			
				MCCMCMINIT	LIACGCGTGC	SACCTOCM
901	TCGTCACA	الانك المحمليك	202			
	AGCAGTGT	CAAAAGAT	TCATAGAT	CAGGCAAAT( GTCCGTTTA(	GAGGTAATTI CTCCATTAA	ACTAATGC

# FIGURE 22D (CONT'D)

951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CITTLETCIGITATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATGATTGCAATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
	CIACIACIACIA TACGITATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGCCCACCATTATACCC
	TGACTACGGTGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTAGATCAGAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGAACACCGGGACGTAGGAGGACCGGTTGCTACCTAC
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
	TARACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA
	CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT
	ACCACTGGGTTTGGTTTATATATATATATATATATATATA
	ACCACTGGGTTTGGTTTATACCAATGGTAATAAAACTATCTGTCTAATGA
1751	CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
	GAGAACTCACACACACACACACACACACACACACACACAC
	THE TOTAL CONTROL OF THE TATE TATE TO THE
1801	GGACATTGTA A ATTTTCTA A CTCA A CTCA A CTCA
	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
	CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG
	ACGTC

# FIGURE 23A



#### SUBSTITUTE SHEET (RULE 26)

FIGURE 23

WT preproricin linker

primer HAV-A1

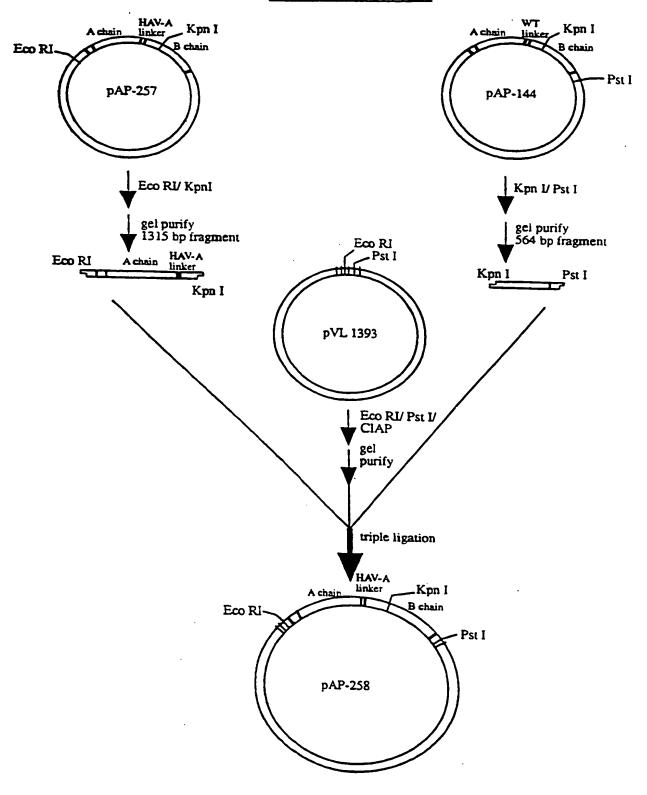
3'- AGCAGTGTCAAAAGACTCGAATCTTGCGTT-5'
primer HAV-A2

PCR mutagenesis
Iigate with pBluescript SK

pAP 257 linker (HAV-A variant) --- TCTGAGCTTAGAACGCAATCGTTCTCAAATTGGAAT ------ AGACTCGAATCTTGCGTTAGCAAGAGTTTAACCTTA -----

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#### FIGURE 23C



SUBSTITUTE SHEET (RULE 26)

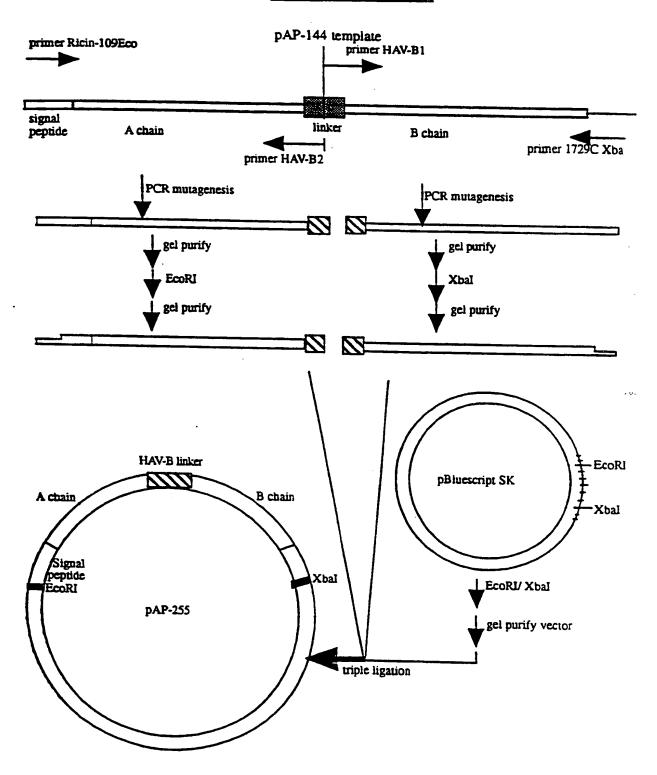
### FIGURE 23D

		10	2	o .	3	0	40	)	50
1	GAATTC	LTGAAACC TACTTTGG	GGGAG	 GAAAT: CTTTA'	ACTAT	   GTAAT   CATT	 ADGTAT	TGTAT	1
E1									
٠.	GGCAACI	ACCGAAA	CAAAA	GGATC( CCTAG(	CACCT( GTGGA(	CAGGG1 GTCCC2	rggtci Accaga	TTCAC AAGTG	ATTAG TAATC
101	AGGATA	CAACATA	TTCCC	CAAAC	AATAC	CCAATI	ATAAA	CTTTA	CCACA
151		TATOTTOT							
	GCGGGTY	GGTGACA	CGTTT	GCTAC. CGATG	ACAAA TGTTT	CTTTA! GAAAT!	CAGAC AGTCTC	CTGTT GACAA	22929 22929
201	TCGTTT:	ACAACTO PTGTTGAC	GAGCT	GATGT CTACA	GAGAC.	ATGAT!	ATACCA	GTGTT	GCCAA
251	ACAGAG:	TGGTTT	CCTAT	אאארר	AACGG	משיע מייניים			
			.GGAIA	11166	TTGCC.	AAATA	<b>LAATCA</b>	LACTTG	AGAGT
301	AATCATO	CAGAGCT	TTCTG	TTACA	TTAGC	GCTGG	ATGTCA	CCAAT	GCATA
351		CGTCTCG							
		CCGATGO	AUJAJ	CCTTT.	ATCGC	GTATAI	<b>LAGAAA</b>	GTAGG	ACTGT
401	ATCAGG:	AGATGCA LTCTACGI	GAAGC CTTCG	AATCA TTAGT	CTCAT	CTTTTC	CACTGA	TGTTC	<b>AAAA</b> T
451	CGATATA	ACATTCGC	CTTTG	GTGGT:	ימידים ב	יים איים איים	~ » ~~~~		
		. GIMGC	GAAAC	CACCA	TTAAT.	ACTATO	TGAAC	TTGTT	Gaacg
501	TGGTAA:	rctgagae Agactcte	TAAAAT	ATCGA TAGCT	GTTGG	GAAAT	GTCCA	CTAGA	GGAGG
551	CTATCT	CAGCGCTT	בידים	מחמתית	מוט ענינים	~~~~			
			MINA!	MAIGT	CATGA	CCACC	STGAGI	CGAAG	GTTGA
601	CTGGCT( GACCGA(	CGTTCCT1 CCAAGGAI	AATAT? TTATA	TTTGC AAACG	ATCCA TAGGT	AATGA? TTACTI	TTTCAG	AAGCA TTCGT	GCAAG CGTTC
651	ATTCCA	ATATATTO	AGGGA	GAAAT	GCGC»	CCACAI			
701			-10001	CILIA	CGCGT	GCTCT!	TAATCO	ATGTT	GGCCT
701	CTAGAC	CACCAGA? STGGTCT!	DATOO! STADO!	CGTAA GCATT	TTACA AATGT	CTTGA(	SARTAC	TTGGG	GGAGA
751	CTTTCC	ACTGCAA1	בע עבודים	N CTICTO					
			210116	I CNGN	TIGGT	TCCTC	<b>GAAAC</b>	GATCA	GGTTA
801	TCAACT	GCAAAGA( CGTTTCT(	GTAAT CATTA	GGTTC	CAAAT GTTTA	TCAGT	STGTAC	GATGT	GAGTA
851	TATTAA	TCCCTAT	CATACC	тстсл	TOOMO	<b></b>			
				WGWG I	ACCAC	ATATC:	TACGCG	TGGAG	GTGGT
901	TCGTCA	CAGTTTT( GTCAAAA(	TGAGC	מטעידיי	20002				
			_		7		<b>~~~</b> (-'1'']	TAACC	אידיוי

# FIGURE 23D (CONT'D)

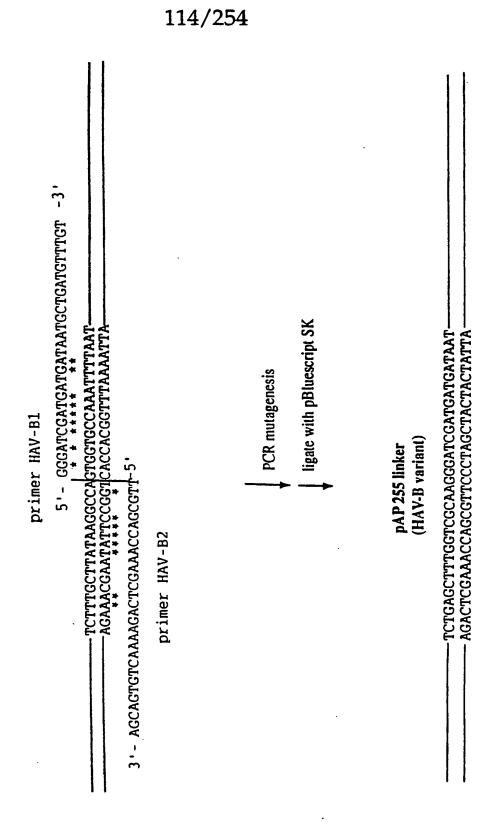
	ESS (COINT D)
951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG
	THE TRACE OF THE PROPERTY OF T
	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCCATCTA
	THE TAXABLE PACKET TO THE TAXABLE PACKET TO THE PACKET TO
	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
	ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC GTCTAGATCAGATC
1301	TTACAGTGCAALCCAACATTTATATTATTATTATTATTATTATTATTATTAT
	THE TACGGLAATCAGTTCCAACCGAAGGATGA
	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTCCCTTAACA
	TGTTAAGATCCTCTCTTCTCCCCCCCCCCCCCCCCCCCC
	THE TACK THE PROPERTY OF THE P
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCCCACCCCACCCCACCCCACCCCACCCCA
	TGGTGACCCAAACCAAATGGGAGAGGT
	THE PROPERTY OF THE PROPERTY O
	CTCTTGCAGTGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTTAAGG
1851	TGCAG ACGTC

#### FIGURE 24A

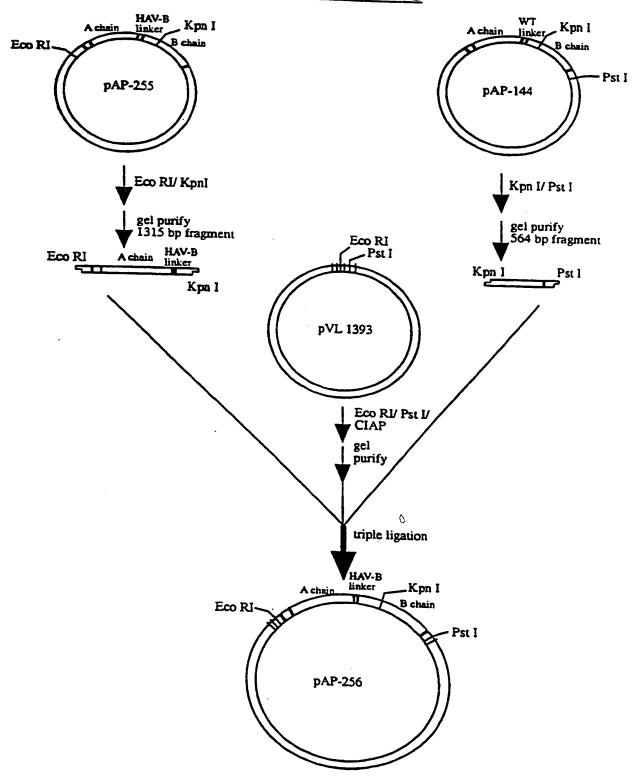


# IGURE 24B

WT preproricin linker



### FIGURE 24C



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# FIGURE 24D

	10	20		30	40	50
1	GAATTCATGAA CTTAAGTACTT	ACCGGGAGG TGGCCCTCC	AAATACTA TTTATGAT	I TTGTAAT; AACATTAT	 LTGGATGTX LACCTACAT	ATGCAGT ACGTCA
51	GGCAACATGGC CCGTTGTACCG	TTTGTTTTG CAAAAAAA	GATCCACC CTAGGTGG	TCAGGGT(	GTCTTTC!	ACATTAG IGTAATC
101	AGGATAACAAC TCCTATTGTTG	ATATTCCCC	AAACAAA			<b></b>
151		TGTGCAAAG	CTACACA 2	3 CTTTT 8 TO		
201	TCGTTTAACAA AGCAAATTGTT	CTGGAGCTG	ATCTCACE	~~~~~~~~		
251	ACAGAGTTGGT TGTCTCAACCA	TTGCCTATA	AACCAACC	مسم دسمیان		
301	AATCATGCAGA TTAGTACGTCT	יפכתתתרתינים.	מידיים מידיים כידיי			
351	TGTGGTCGGCT ACACCAGCCGA	ACCGTGCTG	GAAATAGO			
401		GCAGAAGCA	<b>ユጥ</b> ር ኳርጥር ክ	TOTAL STATE		
451	CGATATACATT	בפככיייייים	יייי א אייי			
501	TGGTAATCTGA	GAGAAA A TA	TCG2CTTA	TACTATO	GAACTTG:	TTGAACG
551	CTATCTCAGCG	CTTTATTAT	TACTCAA(	CCTTTAC	CAGGTGAT	CTCCTCC
	CTGGCTCGTTC	CTTTATAAT	TTGC STCATE	ACCACCG	rgagtega.	aggttga
651			whcg twee	TTTACTA	<i><b>AGTCTTC</b></i>	STCGTTC
	ATTCCAATATA TAAGGTTATAT		. I I I MCGCG	TGCTCTT1	vatccatg:	TTGGCCT
701	CTAGACGTGGT		CMITMATO	TGAACTC	DAADTATT	CCCCTCT
	CTTTCCACTGC GAAAGGTGACC		CMONTIC	ST.LCC.LCC(	SAAACGAT(	CAGGTTA
801	TCAACTGCAA! AGTTGACGTT	GACGTAATO	ביי ביייים	MMC3		
851	TATTAATCCCT ATAATTAGGGI	CATCATACCT	ירייר ז יירכיי			
901	TCGTCACAGT: AGCAGTGTCA	TTCTGAGCT	المستات السالم			

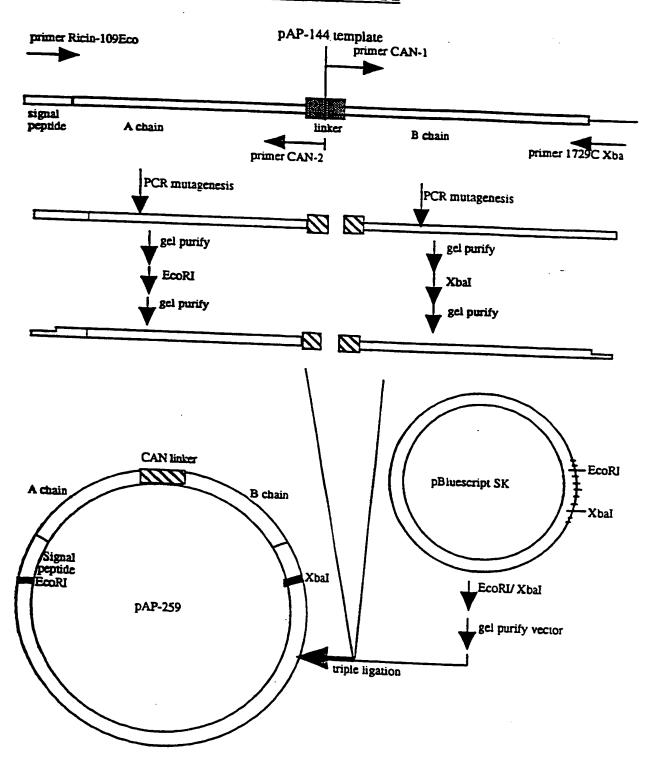
# FIGURE 24D (CONT'D)

951	${f TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATGACTACCATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTACCATAGCATCCAGCTTTACCATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTATAGCATCCAGCTTTACCAGCTAGCATAGCATCCAGCTTTACCAGCTAGAAATGACTACAGCATAGCATCCAGCTTTACCAGCTAGAAATGACAAAACATAACCATAGCATCAGCTTTACCAGCTAGAAATGAAATGAAAAAATGAAAAAAAA$
1001	${\tt GTCTATGTGTTGATGTTAGGGATGGAAGGTTCCACAACGGAAACGCAATACAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT}$
1051	$\tt CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT\\GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA$
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACACGTCTAGATCAGATCAAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	
1751	
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG

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#### FIGURE 25A



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# FIGURE 25E

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	2	<u>ر</u>
ĺ		•
•	3	•

primer can-1	5'- TICAGGCTAAATTTTAATGCTGAT -3'	TCTTTGCTTATAAGGCCAGTGGTGCCAAATTTTAAT——AGAAACGAATATTCGGGTTTAAAATTA——AGAAACGAATATTCGGGTTTAAAATTA——AGAAACGAATATTCGGGTTTAAAATTA——AGAAACGAATATTCGGGTTTAAAATTA——AGAAACGAATATTCGGGTTTAAAATTA——AGAAACGAATATTCGGGTTTAAAATTA——AGAAATTGGGGTTTAAAATTA———AGAAATTGGGGTTTAAAATTA———AGAAACGAATATTCGGGTTTAAAATTA———AGAAATTATGGGGGTTTAAAATTA————————	29.C2 b b b C b b b b C c c c c c c c c c c c
		TCTTPG AGAAC	<b>シャー からつかりずりかりかみをみらかずず</b>

PCR mutagenesis

Ligate with pBluescript SK

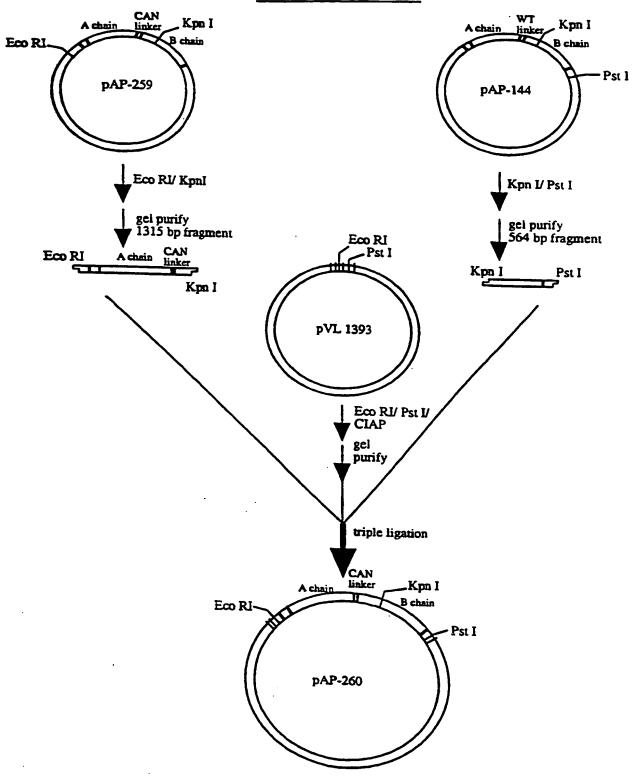
pAP 259 linker (CAN variant) TCTAAGCCTGCAAAGTTCTTCAGGCTAAATTTTAATAGATTCGGACGTTTCAAGAAGTCCGATTTAAAATTA

primer CAN-2

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### FIGURE 25C



SUBSTITUTE SHEET (RULE 26)

# FIGURE 25D

	10	20	30	40	50
1	GAATTCATGAAACC	GGGAGGAAA	TACTATTGT:	AATATGGATGT:	ATGCAGT
	CTTAAGTACTTTGG	CCCTCCTTT	ATGATAACA:	TTATACCTACA:	PACGTCA
51	GGCAACATGGCTTT	GTTTTGGAT	CCACCTCAG	GGTGGTCTTTC:	ACATTAG
	CCGTTGTACCGAAA	CAAAACCTA	GGTGGAGTC	CCACCAGAAAG'	IGTAATC
101	AGGATAACAACATA	TTCCCCAAA	CAATACCCA	ATTATAAACTT	TACCACA
	TCCTATTGTTGTAT	AAGGGGTTT	GTTATGGGT	TAATATTTGAA	ATGGTGT
151	GCGGGTGCCACTGT	GCAAAGCTA	CACAAACTT	TATCAGAGCTG	ITCGCGG
	CGCCCACGGTGACA	CGTTTCGAT	GTGTTTGAA	ATAGTCTCGAC	AAGCGCC
201	TCGTTTAACAACTG	GAGCTGATG	TGAGACATG	ATATACCAGTG	TTGCCAA
	AGCAAATTGTTGAC	CTCGACTAC	ACTCTGTAC	TATATGGTCAC	AACGGTT
251	ACAGAGTTGGTTTG	CCTATAAAC	CAACGGTTT	ATTTTAGTTGA	ACTCTCA
	TGTCTCAACCAAAC	GGATATTTC	GTTGCCAAA	TAAAATCAACT	TGAGAGT
301	AATCATGCAGAGCT	TTCTGTTAC	ATTAGCGCT	GGATGTCACCA	ATGCATA
	TTAGTACGTCTCGA	AAGACAATG	TAATCGCGA	CCTACAGTGGT	TACGTAT
351	TGTGGTCGGCTACC	GTGCTGGAA	ATAGCGCAT	ATTTCTTTCAT	CCTGACA
	ACACCAGCCGATGG	CACGACCTT	TATCGCGTA	TAAAGAAAGTA	GGACTGT
401	ATCAGGAAGATGCA	GAAGCAATO	ACTCATCTT	TTCACTGATGT	TCAAAAT
	TAGTCCTTCTACGT	CTTCGTTAC	TGAGTAGAA	AAGTGACTACA	AGTTTTA
451	CGATATACATTCGC	CTTTGGTGG	TAATTATGA	TAGACTTGAAC	AACTTGC
	GCTATATGTAAGCG	GAAACCACC	ATTAATACT	ATCTGAACTTG	TTGAACG
501	TGGTAATCTGAGAG	AAAATATCO	AGTTGGGAA	ATGGTCCACTA	GAGGAGG
	ACCATTAGACTCTC	TTTTATAGO	TCAACCTT	TACCAGGTGAT	CTCCTCC
551	CTATCTCAGCGCTT	TATTATTAC	AGTACTGGT	GGCACTCAGCT	TCCAACT
	GATAGAGTCGCGAA	ATAATAATC	TCATGACCA	CCGTGAGTCGA	AGGTTGA
601	CTGGCTCGTTCCTT	TATAATTTO	CATCCAAAT	GATTTCAGAAG	CAGCAAG
	GACCGAGCAAGGAA	LATATTAAAC	GTAGGTTTA	CTAAAGTCTTC	GTCGTTC
651	ATTCCAATATATTC	AGGGAGAAI	LTGCGCACGA	GAATTAGGTAC	AACCGGA
	TAAGGTTATATAAC	TCCCTCTT	LTGCTGCTGCT	CTTAATCCATG	TTGGCCT
701	GATCTGCACCAGAT	CCTAGCGT!	TTACACTT	GAGAATAGTTG	GGGGAGA
	CTAGACGTGGTCTA	AGGATCGCA	AADTDTAATT	CAACTATTCTC	CCCCTCT
751	CTTTCCACTGCAA?	TCAAGAGT(	TAACCAAGG	AGCCTTTGCTA	GTCCAAT
	GAAAGGTGACGTTA	AGTTCTCA(	SATTGGTTCC	TCGGAAACGAT	CAGGTTA
801	TCAACTGCAAAGA(	GTAATGGT:	AOTTAAAOO	.GTGTGTACGAT	GTGAGTA
	AGTTGACGTTTCT(	GCATTACCAL	TOAATTTOO	CACACATGCTA	CACTCAT
851	TATTAATCCCTATO ATAATTAGGGATAO	CATAGCTCT( STATCGAGA(	CATGGTGTAT STACCACATA	AGATGCGCACC	TCCACCA AGGTGGT
901	TCGTCACAGTTTTC	CTAAGCCTG(	CAAAGTTCTT	CAGGCTAAATT	TTAATGC
	AGCAGTGTCAAAA	GATTCGGAC(	STTTCAAGAA	GTCCGATTAA	AATTACG

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# FIGURE 25D (CONT'D)

951	${\tt TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATGACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC$
1001	${\tt GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT}$
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACACGTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
1651	GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT
1701	TGGTGACCCAAACCAAATATGGTTACCATTATTTTGATAGACAGATTACT ACCACTGGGTTTGGTTT
1751	CTCTTGCAGTGTGTGTCCTGCCATGAAAATAGATGGCTTAAATAAA
1801	GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
1851	TGCAG ACGTC

### FIGURE 26

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-223/224 linker (MAL-A):

A chain- Q V V Q L Q N Y D E E D -B chain

pAP-225/226 linker (MAL-B):

A chain- L P I F G E S E D N D E -B chain

pAP-227/228 linker (MAL-C):

A chain- Q V V T G E A I S V T M -B chain

pAP-229/230 linker (MAL-D):

A chain- A L E R T F L S F P T N -B chain

pAP-231/pAP-232 linker (MAL-E):

A chain- K F Q D M L N I S Q H Q -B chain

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# FIGURE 27

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-245/246 linker (CMV-A):

A chain- S G V V N A S C R L A N -B chain

pAP-247/248 linker (CMV-B):

A chain- S S Y V K A S V S P E N -B chain

pAP-233/234 linker (HERPES SIMPLEX-1 A):

A chain- S A L V N A S S A H V N -B chain

pAP-235/236 linker (HERPES SIMPLEX-1 B):

A chain- S T Y L Q A S E K F K N -B chain

pAP-249/250 linker (HUMAN HERPES VIRUS-6):

A chain- S S I L N A S V P N F N -B chain

pAP-237/pAP-238 linker (VZV-A):

A chain- S Q D V N A V E A S S N -B chain

pAP-239/pAP-240 linker (VZV-B):

A chain- S V Y L Q A S T G Y G N -B chain

pAP-253/pAP-254 linker (ILV):

A chain- S K Y L Q A N E V I T N -B chain

pAP-255/pAP-256 linker (HAV-A):

A chain- S E L R T Q S F S N W N -B chain

pAP-257/pAP-258 linker (HAV-B):

A chain- S E L W S Q G I D D D N -B chain

### FIGURE 28

Ricin linker (wild type):

A chain- S L L I R P V V P N F N -B chain

pAP-259/260 linker (CAP-A):

A chain- S K P A K F F R L N F N -B chain

pAP-261/262 linker (CAP-B):

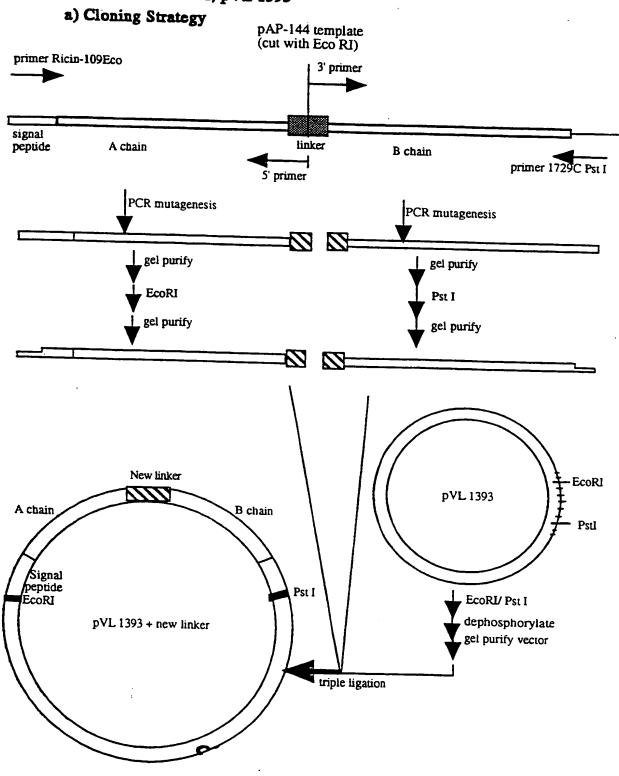
A chain- S K P I E F F R L N F N -B chain

pAP-263/264 linker (CAP-C):

A chain- S K P A E F F A L N F N -B chain

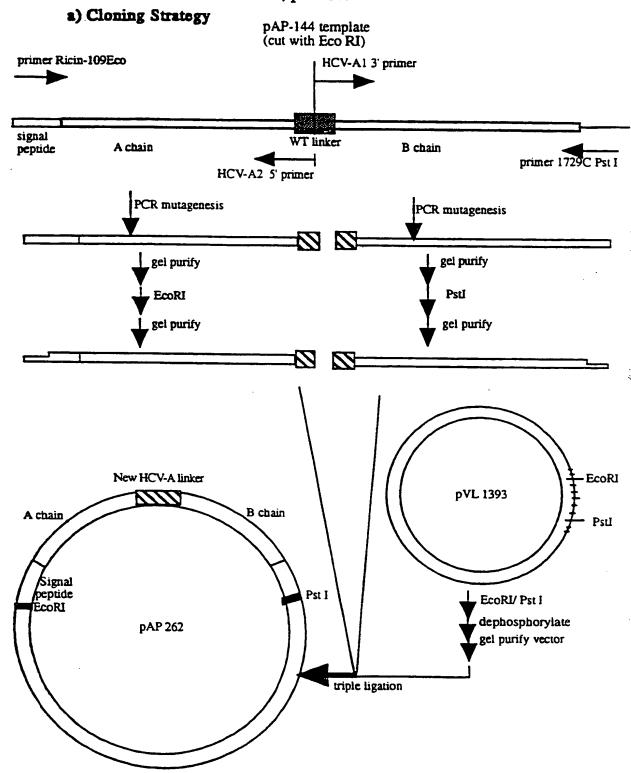
# 126/254 **FIGURE 29**

# PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



### FIGURE 30A

PCR Mutagenesis of Preproricin Gene to Create An HCV-A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# IGURE 30B

# Sequence of HCV-A Linker Region

# WT preproricin linker

primer HCV-A1

5'- TCGACATGGGTTTTTAATGCTGATGTT -3'  *GAAACGAATTTTAATAATTTTAATTTTAATTTTAATTTTAATTTTT	PCR mutagenesis ligate with pVL1393	PAP 262 linker (HCV-A variant)  —GATTTGGAGGTAGTGACATCGACATGGGTTTTTAAT——CTAAACCTCCATCACTGTACCTAACTTTTTAAT——
TCTTT #GAAA GGTAGCAGTGTCAAACTAA 5' pri		GATTTC

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# FIGURE 30C (P1)

### Sequence of pAP262 insert

	10	20	30	40	50
1	GAATTCATGAAACC CTTAAGTACTTTGG	  GGGAGGAAAT  CCCTCCTTTA	 ACTATTGTA: TGATAACAT	 ATATGGATG: TATACCTAC!	i TATGCAGT ATACGTCA
51	GGCAACATGGCTTT CCGTTGTACCGAAA	GTTTTGGATC	CACCTCAGG	<b>ら</b> からなって アイファイン アイフィー アイファイン アイフィイ アイファイン アイファイン アイファイン アイファイン アイファイン アイフィア アイファイ アイフィア アイファイ アイフィー アイフィア アイファイン アイフィア アイファイ アイフィア アイフィア アイファイ アイフィア アイフィア アイフィア アイファイ アイファ アイファイ アイファイン アイファイ アイファイン アイファイン アイファイン アイファイ アイファイ アイファイ アイファイ アイファイ アイファイ アイファイ アイア アイファイ アイファイ アイファイ アイファイ アイファイ アイファイ アイファイ アイファイ アイア アイファイ アイア アイア アイア アイア アイア アイア アイア アイア アイア ア	こみぐみがかみぐ
101	AGGATAACAACATA	TTCCCCAAAC	AATACCCAA	TTATAAACT1	アヤスへへみぐみ
	TCCTATTGTTGTAT	AAGGGGTTTG	TTATGGGTT.	AATATTTGA <i>i</i>	\ATGGTGT
151	GCGGGTGCCACTGT CGCCCACGGTGACA	GCAAAGCTAC CGTTTCGATG	ACAAACTTT. TGTTTGAAA	ATCAGAGCTO TAGTCTCGAO	STTCGCGG CAAGCGCC
201	TCGTTTAACAACTG AGCAAATTGTTGAC	GAGCTGATGT CTCGACTACA	GAGACATGA CTCTGTACT	TATACCAGTO ATATGGTCAC	TTGCCAA AACGGTT
251	ACAGAGTTGGTTTG TGTCTCAACCAAAC	CCTATAAACC	AACGGTTTA'	TTTTAGTTC	א השרשרא
301	AATCATGCAGAGCT	TTCTGTTACA	TTAGCGCTG	GATGTCACCA	አጥር C አጥአ
	TAGTACGTCTCGA	AAGACAATGT	AATCGCGAC	CTACAGTGGI	TACGTAT
351	TGTGGTCGGCTACC ACACCAGCCGATGG	GTGCTGGAAA CACGACCTTT	TAGCGCATA: ATCGCGTATI	ITTCTTTCAT AAAGAAAGTA	CCTGACA GGACTGT
101	ATCAGGAAGATGCA TAGTCCTTCTACGT	GAAGCAATCA CTTCGTTAGT	CTCATCTTT: GAGTAGAAA!	ICACTGATGT AGTGACTACA	TCAAAAT AGTTTTA
151	CGATATACATTCGC GCTATATGTAAGCG	CTTTGGTGGT GAAACCACCA	AATTATGATA TTAATACTA:	AGACTTGAAC ICTGAACTTG	AACTTGC
501	TGGTAATCTGAGAG. ACCATTAGACTCTC	AAAATATCGA	GTTGGGAAA1	<b>ではないしょう アンドゥック アンドゥック アンドゥック アンドゥック アン・アン・アン・アン・アン・アン・アン・アン・アン・アン・アン・アン・アン・ア</b>	CACCACC
551	CTATCTCAGCGCTT	TATTATTACA	GTACTGGTGG	ここないかいないか	שר א א א
501	GATAGAGTCGCGAA CTGGCTCGTTCCTT				
	CTGGCTCGTTCCTT GACCGAGCAAGGAA	ATATTAAACG	TAGGTTTACT	TAAAGTCTTC	GTCGTTC
551	ATTCCAATATATTG. TAAGGTTATATAAC	AGGGAGAAAT TCCCTCTTTA	GCGCACGAG <i>i</i> CGCGTGCTC1	ATTAGGTAC FTAATCCATG	AACCGGA TTGGCCT
701	GATCTGCACCAGAT CTAGACGTGGTCTA	CCTAGCGTAA GGATCGCATT	TTACACTTG! AATGTGAACT	AGAATAGTTG PCTTATCAAC	GGGGAGA
751	CTTTCCACTGCAAT GAAAGGTGACGTTA	TCAAGAGTCT	AACCAAGGAG	こしてなると	CTCCNNT

# FIGURE 30C (P2)

801	TCAACTGCAAAGACGTAATGGTTCCAAATTTCA
	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACACA

- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTGATTTGGAGGTAGTGACATCGACATGGGTTTTTAATGC AGCAGTGTCAAACTAAACCTCCATCACTGTAGCTGTACCCAAAAATTACG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
  GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
  TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAAATTTAAACATATCACCTAACCACAATCTA

### FIGURE 30C (P3)

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP262

### FIGURE 30D

-Amino Acid Sequence Comparison of Mutant Preproricin Linker Region of HCV-A to Wild Type

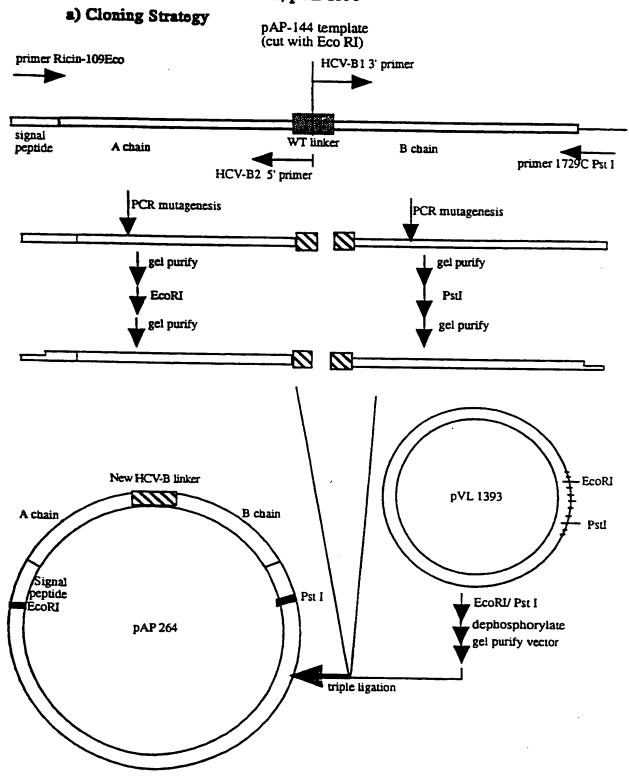
Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-262 linker: (HCV-A linker)

A chain- D L E V V T S T W V F N -B chain

### 133/254 FIGURE 31A

PCR Mutagenesis of Preproricin Gene to Create An HCV-B Variant Gene in Baculovirus Transfer Vector, pVL 1393

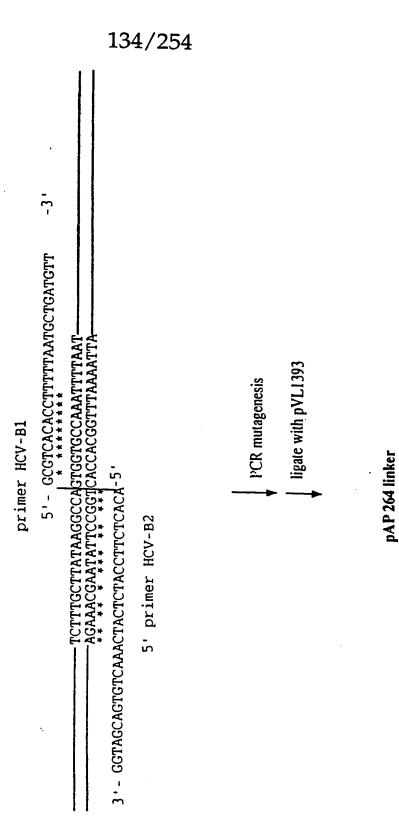


(HCV-B variant)

# IGURE 31B

# Sequence of HCV-B Linker Region

WT preproricin linker



SUBSTITUTE SHEET (RULE 26)

# FIGURE 31C (P1)

Sequence of pAP264 insert

		10	20	30	40	50
		Į	ı	1	1	1
1	GAATTCA	TGAAACCG	GGAGGAAAT	ACTATTGTA	ATATGGAT(	י דמיתכרמכית
	CTTAAGT.	ACTTTGGC	CCTCCTTTA	TGATAACAT	TATACCTA	STATOCACT
				TONIMCAL	INIACCIA	-ATACGTCA
51	GGCAACA	TCCCTTTC	· TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	63.66mas ac		
-	CCCTTCT	16661116	TTTTGGATC	CACCTCAGG	GTGGTCTT	<b>CACATTAG</b>
	CCGTTGT.	ACCGAAAC	AAAACCTAG	GTGGAGTCC	CACCAGAAI	AGTGTAATC
101	AGGATAA	CAACATAI	TCCCCAAAC	AATACCCAA	TTATAAACT	ר ארב אר א
	TCCTATT	GTTGTATA	AGGGGTTTG	TTATEGETT	22T2TTTC	A A TOCTOR
					mini i Gr	MATGGIGI
151	GCGGGTG	CCACTGTG	CAAAGCTAC	7 C 7 7 7 C 7 C 7 C	1 m c 1 c 1 c 1	
	CGCCCAC	GGTGACAC		ACAAACITI.	ATCAGAGC'	rgttcgcgg
	CCCCAC	GGIGACAC	GTTTCGATG	TGTTTGAAA	TAGTCTCG	ACAAGCGCC
201	mocmmes.					
201	TCGTTTA	ACAACTGG	AGCTGATGT	GAGACATGA'	TATACCAGI	GTTGCCAA
	AGCAAAT	TGTTGACC	TCGACTACA	CTCTGTACT	ATATGGTC	CAACGGTT
251	ACAGAGT	TGGTTTGC	CTATAAACC	AACGGTTTA	תיייים איייים	~ N N C T C T C N
	TGTCTCA	ACCAAACG	GATATTTGG	TTCCCT 1 1 M	IIIIMGIIO	MACICICA
			02111111100	TIGCCAMAT	AAAATCAAC	TTGAGAGT
301	AATCATG	CAGACCTT	·mcmcmm, c.,			
JU1	TELLCAIG	CAGAGCII	TCTGTTACA	TTAGCGCTG	GATGTCACO	CAATGCATA
	IIAGIAC	GICICGAA	AGACAATGT.	AATCGCGAC	CTACAGTG	TTACGTAT
351	TGTGGTC	GGCTACCG	TGCTGGAAA	TAGCGCATA'	TTTCTTTC	TCCTGACA
	ACACCAG	CCGATGGC	ACGACCTTT.	ATCGCGTAT	AAAGAAAGT	ACCACTOR
						. NOONCIGI
401	ATCAGGA	AGATGCAG	AAGCAATCA	רייים ערייים איני	TC > CTC > TC	
	TAGTCCT	TCTACGTC	TTTCCTTTCA	CICAICIII.	CACTGATO	TTCAAAAT
		-01110010	TTCGTTAGT	GAGTAGAAA	AGTGACTAC	AAGTTTTA
451	CCNTNTN	~ x mm~~~~				
401	CGRIRIA	CATICGCC	TTTGGTGGT.	AATTATGAT:	<b>AGACTTGA</b> A	CAACTIGC
	GCTATAT	GTAAGCGG	AAACCACCA	TTAATACTA:	TCTGAACTI	GTTGAACG
501	TGGTAAT	CTGAGAGA	AAATATCGA	GTTGGGAAA	TECTOR	יאכאכראכר
	ACCATTA	GACTCTCT	TTTATAGCT	CAACCCTTT		TCTCCTCC
				J. 4.0001111	NCCNGG1GA	riciccicc
551	CTATCTC	AGCGCTTT	. ארי א יחידה א יחידה א			
	GATAGAG	TCCCCDDD	ATTATTACA	GIACTGGTG(	CACTCAGO	TTCCAACT
	Uning No.	LCGCGAAA	TAATAATGT	CATGACCAC	CGTGAGTCG	AAGGTTGA
601	CTCCCTC					
90 I	CIGGCIC	STICCTIT	ATAATTTGC	ATCCAAATG	ATTTCAGAA	GCAGCAAG
	GACCGAG	CAAGGAAA	TATTAAACG	TAGGTTTAC	TAAAGTCTT	CGTCGTTC
651	ATTCCAA!	TATATTGA	GGGAGAAAT	GCGCACGAG	מידים ברידים ב	CNACCCCN
	TAAGGTT	ATATAACT	CCCTCTTTA	CCCCTCCTC	TODALIA	CAACCGGA
			OGCICITIA	cacaracic.	TAATCCAT	GTTGGCCT
701	GATOTOO	ACCAGATO	CT3 CCC55	Mm. a. a		
	CTACACC		CTAGCGTAA'	TTACACTTG	AGAATAGTT	'GGGGGAGA
	CINGACG.	TGGTCTAG	GATCGCATT	AATGTGAAC:	ICTTATCAA	CCCCCTCT
751						
121	CTTTCCA	CTGCAATT	CAAGAGTCT	AACCAAGGA	SCCTTTGCT	AGTCCAAT
	GAAAGGT	GACGTTAA	GTTCTCAGA	TTGGTTCCTC	CCAAACCA	TCICCOMP

# FIGURE 31C (P2)

801	
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCA
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACC
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGG
901	
	AGCAGTGTCAAACTACTCTACCTTCTCACACGCAGTGTGGAAAAATTACC
951	
	ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	
	CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTA
3051	
1021	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTT
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	
1101	
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATG
1151	
	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
	CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC
	GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1000	
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
7757	
T221	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
3401	
	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	
	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
	TOACIAAGAI IATATGCCCTTTGTCA

1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT

AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

### FIGURE 31C (P3)

- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP264

# FIGURE 31D

-Amino Acid Sequence Comparison of Mutant Preproricin Linker Region of HCV-B to Wild Type

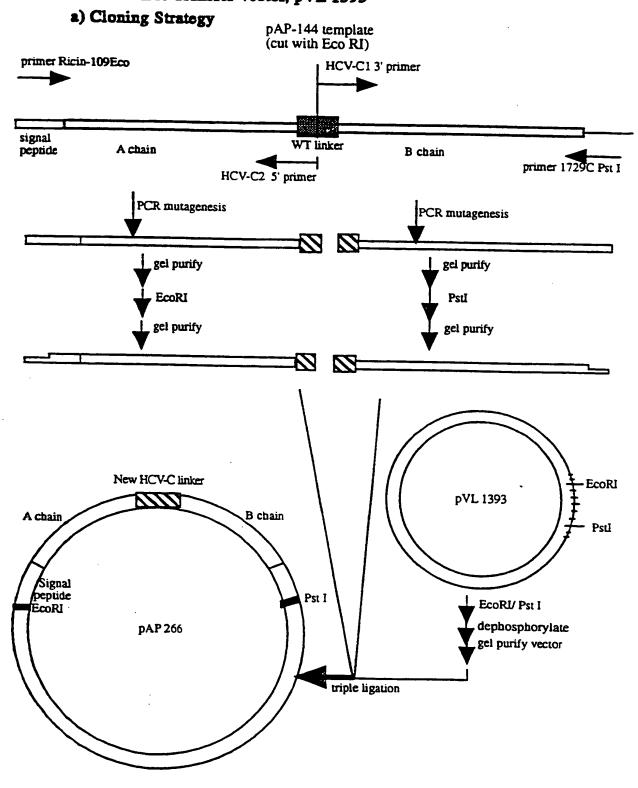
Wild type Ricin linker: A chain-SLLIRPVVPNFN-B chain

pAP-264 linker: (HCV-B linker)

A chain- D E M E E C A S H L F N -B chain

### FIGURE 32A

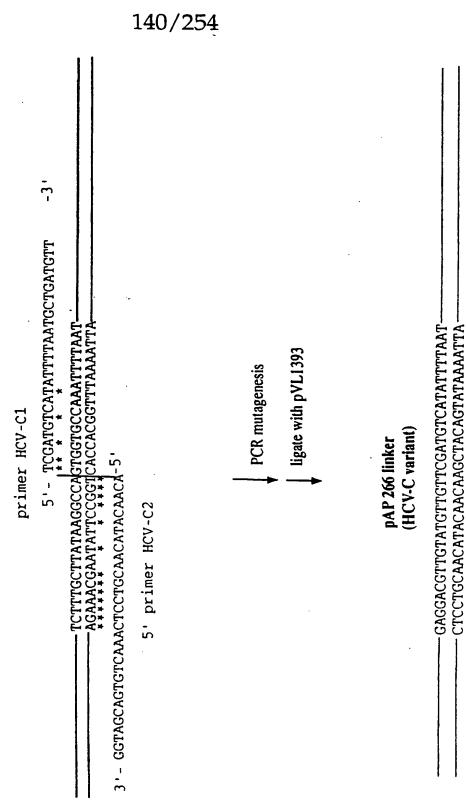
- PCR Mutagenesis of Preprozicin Gene to Create An HCV-C Variant Gene in Baculovirus Transfer Vector, pVL 1393



# FIGURE 32B

# Sequence of HCV-C Linker Region

# WT preproricin linker



# FIGURE 32C (P1)

#### Sequence of pAP266 insert

	10	20	30	40	50
1	GAATTCATGAAAC CTTAAGTACTTTG				
51	GGCAACATGGCTT	TGTTTTGGATC	CACCTCAGG	STGGTCTTTC	ACATTAG
	CCGTTGTACCGAA	ACAAAACCTAG	GTGGAGTCC	CACCAGAAAG	TGTAATC
101	AGGATAACAACAT	ATTCCCCAAAC	AATACCCAA?	ITATAAACTT	TACCACA
	TCCTATTGTTGTA	TAAGGGGTTTG	TTATGGGTT?	AATATTTGAA	ATGGTGT
151	GCGGGTGCCACTG	TGCAAAGCTAC	ACAAACTTT	ATCAGAGCTG	TTCGCGG
	CGCCCACGGTGAC	ACGTTTCGATG	TGTTTGAAA	FAGTCTCGAC	AAGCGCC
201	TCGTTTAACAACT AGCAAATTGTTGA				
251	ACAGAGTTGGTTT TGTCTCAACCAAA	GCCTATAAACC.	AACGGTTTA: TTGCCAAAT	TTTAGTTGA AAAATCAACT	ACTCTCA TGAGAGT
301	AATCATGCAGAGO	TTTCTGTTACA	TTAGCGCTG(	GATGTCACCA	ATGCATA
	TTAGTACGTCTCG	AAAGACAATGT	AATCGCGAC(	CTACAGTGGT	TACGTAT
351	TGTGGTCGGCTAC	CGTGCTGGAAA	TAGCGCATA:	ITTCTTTCAT	CCTGACA
	ACACCAGCCGATG	GCACGACCTTT	ATCGCGTATA	AAAGAAAGTA	GGACTGT
401	ATCAGGAAGATGO	AGAAGCAATCA	CTCATCTTT:	ICACTGATGŤ	TCAAAAT
	TAGTCCTTCTACG	TCTTCGTTAGT	GAGTAGAAA	AGTGACTACA	AGTTTTA
451	CGATATACATTCG	CCTTTGGTGGT	AATTATGATA	AGACTTGAAC	AACTTGC
	GCTATATGTAAGC	GGAAACCACCA	TTAATACTA	ICTGAACTTG	TTGAACG
501	TGGTAATCTGAGA	GAAAATATCGA	GTTGGGAAA'	IGGTCCACTA	GAGGAGG
	ACCATTAGACTCT	CTTTTATAGCT	CAACCCTTT	ACCAGGTGAT	CTCCTCC
551	CTATCTCAGCGCT	TTATTATTACA	GTACTGGTGG	GCACTCAGCT	TCCAACT
	GATAGAGTCGCGA	AATAATAATGT	CATGACCAC	CGTGAGTCGA	AGGTTGA
601	CTGGCTCGTTCCT	TTATAATTTGC	ATCCAAATG:	ATTTCAGAAG	CAGCAAG
	GACCGAGCAAGG	AATATTAAACG	TAGGTTTAC	IAAAGTCTTC	GTCGTTC
651	ATTCCAATATAT?	GAGGGAGAAAT	GCGCACGAG.	AATTAGGTAC	AACCGGA
	TAAGGTTATATA?	CTCCCTCTTA	CGCGTGCTC	TTAATCCATG	TTGGCCT
701	GATCTGCACCAGA	ATCCTAGCGTAA	TTACACTTG.	AGAATAGTTG	GGGGAGA
	CTAGACGTGGTCT	PAGGATCGCATT	'AATGTGAAC	TCTTATCAAC	CCCCTCT
751	CTTTCCACTGCAL GAAAGGTGACGT	ATTCAAGAGTCT	AACCAAGGA	GCCTTTGCTA	GTCCAAT

### FIGURE 32C (P2)

	FIGURE 32C (P2)
801	
	AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
851	
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
901	
	AGCAGTGTCAAACTCCTGCAACATACAACAAGCTACAGTATAAAATTACG
951	
	ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA
	CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	
	GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
7757	
1151	
	CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	
1201	ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
	TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	
1201	
	GTCTAGATCAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	
2001	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
	AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	
	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
	TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	
	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA
	GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACACTCCCCTCTTTTTTTTTTTTTTTTTT
	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
	TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GTTTTGGCTCTATTAACGGAATCTTCACTTCACTTCACT
	GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
	ACAATTCTAGGAGAGAACACCGGGACGTAGGAGACCGGTTGCTACCTAC
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT
	AGTTCTTACTACCTTGGTAAAATTTTAAACATTGGTGTTAGAT

AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA

### FIGURE 32C (P3)

- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP266

# FIGURE 32D

-Amino Acid Sequence Comparison of Mutant Preproricin Linker Region of HCV-C to Wild Type

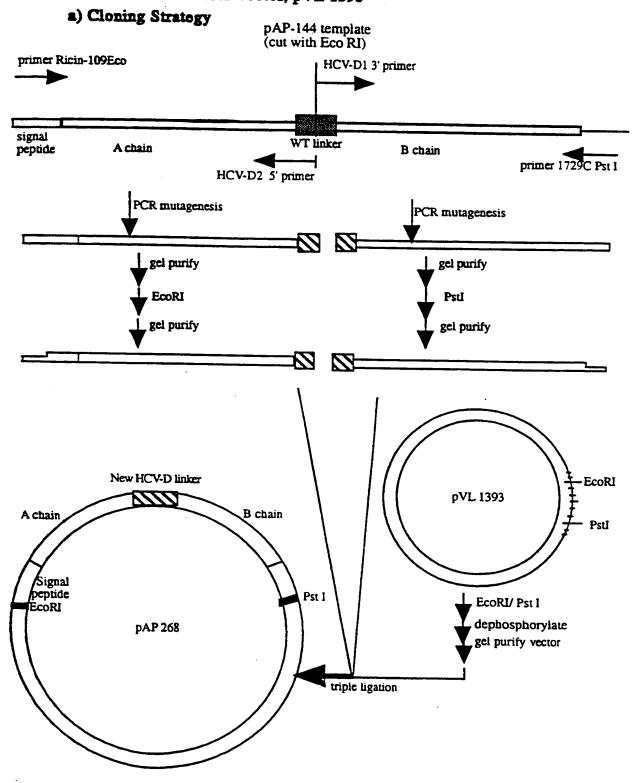
Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-266 linker: (HCV-C linker)

A chain- E D V V C C S M S Y F N -B chain

### FIGURE 33A

PCR Mutagenesis of Preproricin Gene to Create An HCV-D Variant Gene in Baculovirus Transfer Vector, pVL 1393



- AAGGGGTGGAGATTGCTAGCGCCAATAACTGCTTAT-· TTCCCCCACCTCTAACGATCGCGGTTATTGACGAATA-

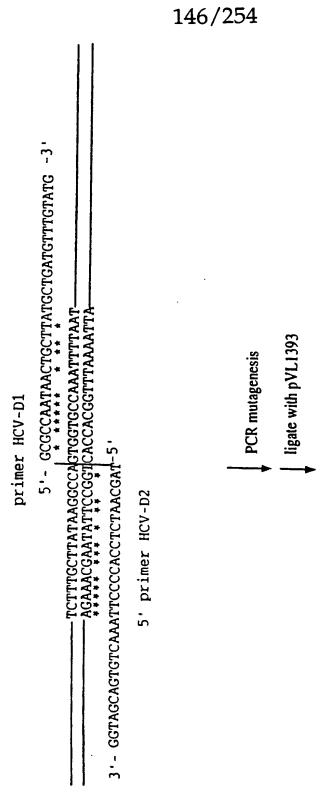
(HCV-D variant)

pAP 268 linker

# FIGURE 331

Sequence of HCV-D Linker Region

# WT preproricin linker



# FIGURE 33C (P1)

#### Sequence of pAP268 insert

	1	.0	20	30	40	50
1	GAATTCATO CTTAAGTAC	AAACCGGG TTTGGCCC	aggaaat Cootta	ACTATTGT TGATAACA	AATATGGAT TTATACCTA	I GTATGCAGT CATACGTCA
51	GGCAACATO	GCTTTGTT	TTGGATO	CACCTCAG	GGTGGTCTT	TCACATTAG
	CCGTTGTAC	CGAAACA	AAACCTAO	GTGGAGTC	CCACCAGAA	AGTGTAATC
101	AGGATAACA	ACATATTO	CCCAAAC	AATACCCA	ATTATAAAC	TTTACCACA
	TCCTATTGT	TGTATAAO	GGGTTTC	STTATGGGT	TAATATTTO	SAAATGGTGT
151	GCGGGTGCC	ACTGTGC!	AAAGCTAC	ACAAACTT	TATCAGAGO	TGTTCGCGG
	CGCCCACGG	STGACACG	TTTCGATC	STGTTTGAA	ATAGTCTCO	SACAAGCGCC
201	TCGTTTAAC	CAACTGGA(	CTGATG1	GAGACATG	ATATACCAG	STGTTGCCAA
	AGCAAATTC	STTGACCT(	CGACTAC	CTCTGTAC	TATATGGTC	CACAACGGTT
251	ACAGAGTTO	GTTTGCC1	TATÁAACO	CAACGGTTT	ATTTTAGTI	GAACTCTCA
	TGTCTCAAC	CAAACGG1	TATTTGO	STTGCCAAA	TAAAATCAA	CTTGAGAGT
301	AATCATGCA TTAGTACGT	GAGCTTT(	TGTTACA SACAATG1	TTAGCGCT AATCGCGA	GGATGTCAC CCTACAGTG	CAATGCATA GTTACGTAT
351	TGTGGTCGG ACACCAGCG	GATGGCA	GACCTT	TAGCGCAT TATCGCGTA	ATTTCTTTC	ATCCTGACA STAGGACTGT
401	ATCAGGAAC	SATGCAGAI	AGCAATC!	ACTCATCTT	TTCACTGAT	GTTCAAAAT
	TAGTCCTTC	CTACGTCT:	CGTTAGT	GAGTAGAA	AAGTGACTA	CAAGTTTTA
451	CGATATACA	ATTCGCCT:	TTGGTGG:	raattatga	TAGACTTGA	ACAACTTGC
	GCTATATG	PAAGCGGAL	AACCACC	Ytaatact	ATCTGAACT	TGTTGAACG
501	TGGTAATC!	GAGAGAA CTCTCTT!	AATATCG! !TATAGC!	AGTTGGGAA TTJJJAAJ1	ATGGTCCAC	CTAGAGGAGG SATCTCCTCC
551	CTATCTCA( GATAGAGT(	GCGCTTTA'	TATTACI AATAATG:	AGTACTGGT CATGACCA	GGCACTCAG CCGTGAGTO	SCTTCCAACT SGAAGGTTGA
601	CTGGCTCG:	TCCTTTA'	TAATTTG(	CATCCAAAT	GATTTCAGA	AGCAGCAAG
	GACCGAGC	AAGGAAAT	ATTAAAC(	STAGGTTTA	CTAAAGTC1	TCGTCGTTC
651	ATTCCAAT	ATATTGAG IATAACTC	GGAGAAA' CCTCTTT	rgcgcacga ACGCGTGCT	GAATTAGG1	racaaccgga Atgttggcct
701	GATCTGCA	CCAGATCC	TAGCGTA	ATTACACTT	GAGAATAG?	TTGGGGGAGA
	CTAGACGT	GGTCTAGG	ATCGCAT	FAATGTGAA	CTCTTATC!	ACCCCCTCT
751	CTTTCCAC	TGCAATTC	AAGAGTC	TAACCAAGG	SAGCCTTTG(	CTAGTCCAAT
	GAAAGGTG	ACGTTAAG	TTCTCAG	ATTGGTTCC	CTCGGAAAC(	GATCAGGTTA

# FIGURE 33C (P2)

801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTAAGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
	ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
901	TCGTCACAGTTTAAGGGGTGGAGATTGCTAGCGCCAATAACTGCTTATGCAGCAGTGTCAAATTCCCCACCTCTAACGATCGCGGTTATTGACGAATACG
951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGCTCCAAA
	ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG
	CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACACTCCTAGGA
	GICIAGAICAGATCAAAATCGTCGCTGTAGTCCCTTGTCACCATGGTGTG
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT
	GITTIGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
1551	TGTTAAGATCCTCTTGTGGCCCTGCATCCTCTGGCCAACGATGGATG
1601	TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTCTTTAGA
	AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACATTCTTA

# FIGURE 33C (P3)

- 1651 GTGAGGCGATCGGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP268

# FIGURE 33D

-Amino Acid Sequence Comparison of Mutant Preproricin Linker Region of HCV-D to Wild Type

Wild type Ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-268 linker: (HCV-D linker)

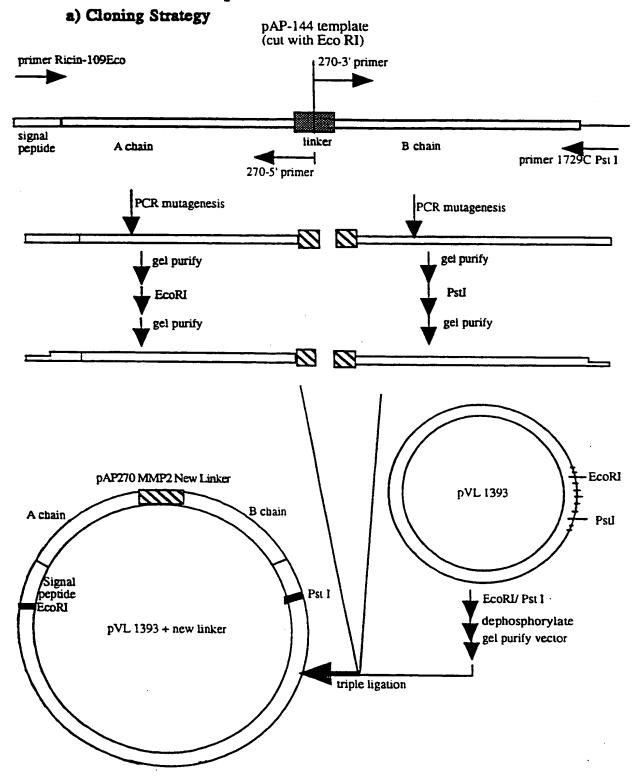
A chain- K G W R L L A P I T A Y -B chain

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### FIGURE 34A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# FIGURE 34B

### Sequence of MMP-2 Linker Region

### WT preprocin linker

primer	270-3			
	TGGGCTCCTAATTTTAATGCTGATGTTTGT -3'			
TCTTTGCTTATAAGGCCA	GTGGTACCAAATTTTAAT			
AGAAACGAATATTCCGGT	CACCATGGTTTAAAATTA			
3'-AGCAGTGTCAAAAGAAACGGGGACCCAAAT primer 270-5'	-5'			
1) PCR n	nutagenesis			
2) Ligate with pVL1393				
pAP 270 (MMP-2	linker variant)  TGGGCTCCTAATTTTAAT			
AGAAACGGGGACCCAAAT	ACCCGAGGATTAAAATTA			

## FIGURE 34C (P1)

Sequence of pAP270 insert

	10	20	30	40	50
1	GAATTCATGAAACCG CTTAAGTACTTTGGC				
51	GGCAACATGGCTTTG CCGTTGTACCGAAAC				
101	AGGATAACAACATAT	TCCCCAAAC	CAATACCCAA	TTATAAACTT	TACCACA
	TCCTATTGTTGTATA				
151	GCGGGTGCCACTGTG CGCCCACGGTGACAC				
201	TCGTTTAACAACTGG				
251	ACAGAGTTGGTTTGC				
221	ACAGAGTTGGTTTGC TGTCTCAACCAAACG				
301	AATCATGCAGAGCTT TTAGTACGTCTCGAA	TCTGTTACA AGACAATGT	ATTAGCGCTG TAATCGCGAC	GATGTCACCA CTACAGTGGT	ATGCATA
351	TGTGGTCGGCTACCG	TGCTGGAAA	ATAGCGCATA	TTTCTTTCAT	rcctgaca
407	ACACCAGCCGATGGC				
401	ATCAGGAAGATGCAG TAGTCCTTCTACGTC	AAGCAATCA TTCGTTAGI	ACTCATCTTT GAGTAGAAA	TCACTGATGT AGTGACTAC	TTCAAAAT AGTTTTA
451	CGATATACATTCGCC GCTATATGTAAGCGC	TTTGGTGGT	TAATTATGAT ATTAATACTA	AGACTTGAAC	CAACTTGC
501	TGGTAATCTGAGAGA	AAATATCGA	AGTTGGGAAA	TGGTCCACT	AGAGGAGG
	ACCATTAGACTCTCT				
221	CTATCTCAGCGCTTT GATAGAGTCGCGAAA	TATTATTACI TAATAATGI	AGTACTGGTG FCATGACCAC	GCACTCAGC: CGTGAGTCG	ITCCAACT AAGGTTGA
601	CTGGCTCGTTCCTT: GACCGAGCAAGGAA	TATAATTTG(	CATCCAAATC	SATTTCAGAA(	GCAGCAAG
651	ATTCCAATATATTG				
	TAAGGTTATATAAC	CCCTCTTT	ACGEGTECT	TTARTECATE	STTGGCCT

# 154/254 FIGURE 34C (P2)

- 701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
  AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- B51 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTTCTTTGCCCCTGGGTTTATGGGCTCCTAATTTTAATGC AGCAGTGTCAAAAGAAACGGGGGACCCAAATACCCGAGGATTAAAATTACG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
  GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
  AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT

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### FIGURE 34C (P3)

- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP270

### FIGURE 34D

Amino acid sequence Comparison of Mutant Preproricin Linker region of MMP-2 to Wild Type

Wild type ricin linker:

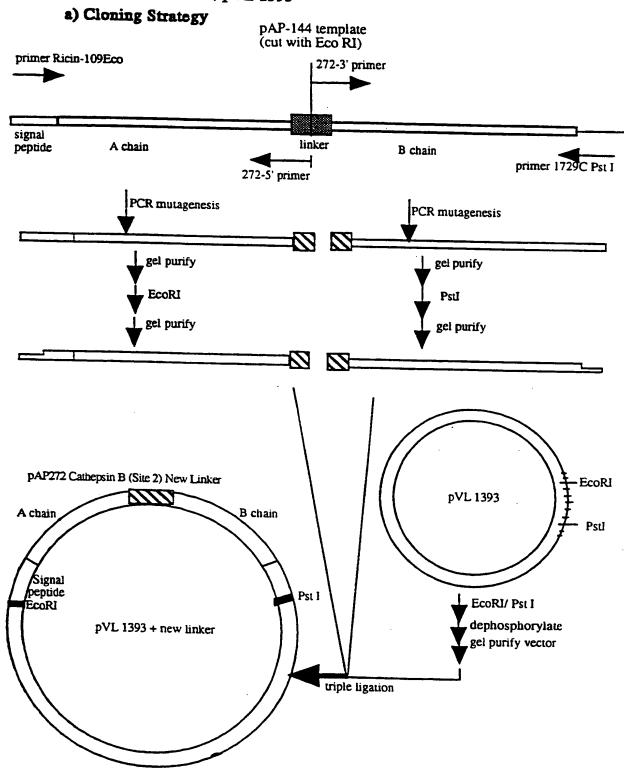
A chain- S L L I R P V V P N F N -B chain

pAP-270 (MMP-2) linker:

A chain- S L P L G L W A P N F N -B chain

#### FIGURE 35A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



### FIGURE 35B

### Sequence of Cathepsin B (Site 2) Linker Region

#### WT preprocin linker

primer	272-3'
	AGGATGCCAAATTTTAATGCTGATGTTTGT -3'
TCTTTGCTTATAAGGCCAAGAAACGAATATTCCGGT	GTGGTACCAAATTTTAAT
3'-AGCAGTGTCAAAAGAAACGAATATCGATCT primer 272-5'	-5'
1) PCR m	nutagenesis
2) Ligate	with pVL1393
TCTTTGCTTATAGCTAGA	in B Site 2 variant) AGGATGCCTAATTTTAAT
AGAAACGAATATCGATCT	TCCTACCCATTA A A A TOTAL

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### 159/254 FIGURE 35C (P1)

#### Sequence of pAP272 insert

	10	20	)	30	40	50
1	רב א איייירא ייירא <del> </del>	7700000			1	
	GAATTCATGA	MACCGGGAGG	SAAATACT	ATTGTAA	TATGGATGT	ATGCAGT
	CIIMGIACI	TTGGCCCTCC	-111AIGA	TAACATT	ATACCTACA	TACGTCA
51	GGCAACATGG	CTTTGTTTT	GATCCAC	CTCAGGG	TCCTCTTTC	
	CCGTTGTACC	GAAACAAAA	CTAGGTG	CI CAGGG GAGTCCC	ACCACAAAC	ACATTAG
					ACCAGAAAG	IGIAATC
101	AGGATAACAA	CATATTCCCC	CAAACAAT	ACCCAAT	TATAAACTT	TACCACA
	TCCTATTGTT	GTATAAGGG	STTTGTTA	TGGGTTA	ATATTTGAA	ATGGTGT
151	GCGGGTGCCA	CTGTGCAAAC	CTACACA	AACTTTA	TCAGAGCTG	TTCGCGG
	CGCCCACGGT	GACACGTTTC	GATGTGT	TTGAAAT.	AGTCTCGAC	AAGCGCC
201	דרקייים א רא	ACTICIA COMO	7 TOTO 2 C			
	TCGTTTAACA AGCAAATTGT	TGACCTCGAC	TALGIGAG.	ACATGAT.	ATACCAGTG	TTGCCAA
		1 Chicci Cont	- INCACIC	IGIACTA	TATGGTCAC	AACGGTT
251	ACAGAGTTGG	TTTGCCTATA	AACCAAC	ᠳ᠘ᠽᠽᡙᢒᢒ	תייים איים איים איים	N CMCMCN
	TGTCTCAACC	AAACGGATAT	TTGGTTG	CCAAATA	TILGIIGA TO A O TA A A A	TCACACT
301	AATCATGCAG	AGCTTTCTGT	TACATTA	GCGCTGG.	ATGTCACCA	ATGCATA
	TTAGTACGTC	TCGAAAGAC	ATGTAAT	CGCGACC	TACAGTGGT	TACGTAT
351	TCTCCTCCC					
221	ACACCACCCC	TACCGTGCTC	GAAATAG	CGCATAT	TTCTTTCAT	CCTGACA
	ACACCAGCCG	ATGGCACGA	CTTTATC	GCGTATA	AAGAAAGTA	GGACTGT
401	ATCAGGAAGA	TGCAGAAGC	א זייר א כיזיר <i>א</i>	سسسسس بن لا	C2 CDC2 ====	
	TAGTCCTTCT	ACGTCTTCGT	TAGTGAG	TACAAAA	CACTGATGT CTC2 CT2 C2	TCAAAAT
				TITOLD TO	GIGACIACA	AGTTTTA
451	CGATATACAT	TCGCCTTTGC	STGGTAAT	TATGATA	GACTTGAAC	A
	GCTATATGTA	AGCGGAAAC	CACCATTA	ATACTAT	CTGAACTTG	TTGAACG
501	TGGTAATCTG	AGAGAAAATI	ATCGAGTT	GGGAAAT	GGTCCACTA	GAGGAGG
	ACCATTAGAC	TCTCTTTTA	TAGCTCAA	CCCTTTA	CCAGGTGAT	CTCCTCC
551	ריים יירייר <i>א</i> כיר	് ് സസസസ സസമ ല	nm> c> c=-			
	CTATCTCAGO	CCITIATIA	TACAGTA	CTGGTGG	CACTCAGCT	TCCAACT
		CGAAATAAT	ATGICAT	GACCACC	GTGAGTCGA	AGGTTGA
601	CTGGCTCGTT	CCTTTATAA	TTGCATC	רא א אדוכה א	プラフィン・フィン・フィン・フィン・フィン・フィン・フィン・フィン・フィン・フィン・	C)
	GACCGAGCAZ	AGGAAATATTI	AAACGTAG	GTTTACT	IIICAGAAG AAAGTCTTC	CAGCAAG
_						
651	ATTCCAATAT	TATTGAGGGA	GAAATGCG	CACGAGA	ATTAGGTAC	AACCGGA
	TAAGGTTAT	ATAACTCCCT	CTTTACGC	GTGCTCT	TAATCCATG	TTGGCCT

#### 160/254 FIGURE 35C (P2)

701	CATCTCCACCACACACACACACACACACACACACACACA	•
, 0 1	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGA	~ ~
	CTACA CCTCCTCTA COL TOTAL	GA
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCT	
		t I

- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- B01 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTTCTTTGCTTATAGCTAGAAGGATGCCTAATTTTAATGC AGCAGTGTCAAAAGAAAGGAATATCGATCTTCCTACGGATTAAAATTACG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
  GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT

### FIGURE 35C (P3)

- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP272

# FIGURE 35D

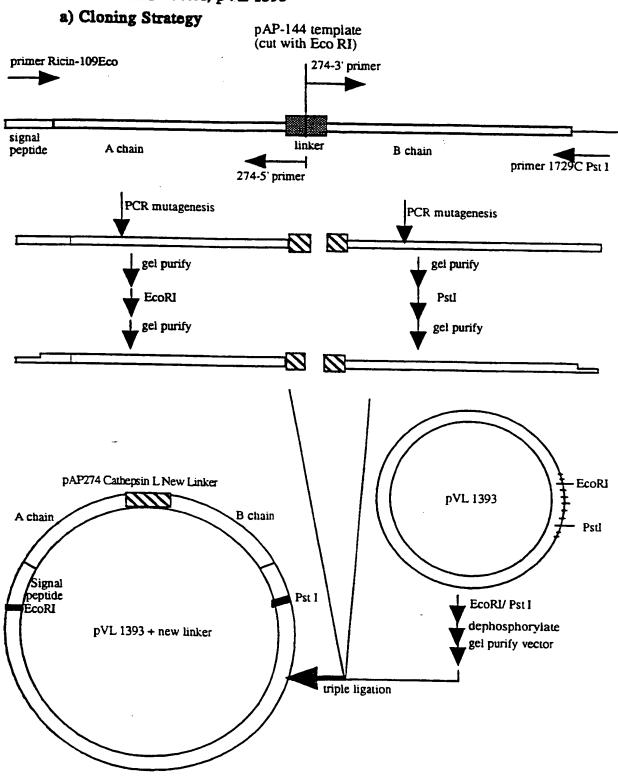
Amino acid sequence Comparison of Mutant Preproricin Linker region of Cathepsin B Site 2 to Wild Type

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-272(Cathepsin B 2)linker: A chain- S L L I A R R M P N F N -B chain

#### FIGURE 36A

# PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



#### FIGURE 36B

### Sequence of Cathepsin L Linker Region

#### WT preprocin linker

	TCATGGGCTAATTTTAATGCTGATGTTTGT -3'
	GTGGTACCAAATTTTAAT
3'-AGCAGTGTCAAAAGAAACGAATATAAGGCC primer 274-5'	-5'
1) PCR n	nutagenesis
2) Ligate	with pVL1393
pAP 274 (CathepsTCTTTGCTTATATTCCGG	sin L variant)  TCATGGGCTAATTTTAAT

# 165/254 FIGURE 36C (P1)

#### Sequence of pAP274 insert

	10	20	30	40	50
٦.	- CDDTTCDTCDDDC				ł
-	GAATTCATGAAAC	~GGGAGGA <u>AA;</u> ~CCCTCCTTTT	TACTATTGTA	ATATGGATG	TATGCAGT
	CTTAAGTACTTTG	30001001112	AIGATAACAT	TATACCTAC	ATACGTCA
51	GGCAACATGGCTT	TGTTTTGG&T(	CCD CCTCD CC		
	CCGTTGTACCGAA	ACAAAACCTAG	GCTGGAGTCC	CACCACAAA	CACATTAG
	·			CACCAGAAA	GIGIAATC
101	AGGATAACAACAT	ATTCCCCAAA	CAATACCCAA	ТТАТАААСТ	ידידמ ררמ רמ
	TCCTATTGTTGTA	<b>FAAGGGGTTT</b>	GTTATGGGTT	AATATTTGA	AATGGTGT
151	GCGGGTGCCACTG	rgcaaagcta(	CACAAACTTI	ATCAGAGCT	'GTTCGCGG
	CGCCCACGGTGAC	ACGTTTCGAT	GTGTTTGAAA	TAGTCTCGA	CAAGCGCC
222	maammin n an n an				
201	TCGTTTAACAACT	GGAGCTGATG	IGAGACATGA	TATACCAGT	GTTGCCAA
	AGCAAATTGTTGA	CCTCGACTAC	ACTCTGTACT	TATATGGTCA	.CAACGGTT
251	ACAGAGTTGGTTT	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	73 3 GGGGGGG		
	ACAGAGTTGGTTT(	CCIAIAAAC(	LAACGGTTTA	TTTTAGTTG	AACTCTCA
	TGTCTCAACCAAA	COGRIFIE	31 1GCCAAA1	AAAATCAAC	TTGAGAGT
301	AATCATGCAGAGC:	TTTCTGTTAC	איייאפרפריים איייאפרפריים	באתרתרא הר	13 3 MC C3 M3
	TTAGTACGTCTCG	AAAGACAATG	PAATCGCGAC	CTACACTCC	AATGCATA
				.e.rheng166	TIACGIAI
351	TGTGGTCGGCTAC	CGTGCTGGAAL	ATAGCGCATA	TTTCTTTCA	ТССТСАСА
	ACACCAGCCGATG	GCACGACCTT.	TATCGCGTAT	AAAGAAAGT	'AGGACTGT
401	ATCAGGAAGATGC	AGAAGCAATC	ACTCATCTTI	TCACTGATG	TTCAAAAT
	TAGTCCTTCTACG	rcttcgttag:	<b>IGAGTAGAAA</b>	AGTGACTAC	'AAGTTTTA
451					
3 D T	CGATATACATTCG(	CCTTTGGTGG;	PAATTATGAT	AGACTTGAA	.CAACTTGC
	GCTATATGTAAGC	GAAACCACC	ATTAATACTA	TCTGAACTT	'GTTGAACG
501	TGGTAATCTGAGA	בא א א שר א מיניים מיניים מיניים מיניים		maamaas am	
	ACCATTAGACTCT	CTTTTATAGC'	TCD D CCCTTT	TIGGTCCACT	AGAGGAGG
			COMCCCIII	.ACCAGGTGA	rerectee
551	CTATCTCAGCGCT'	TTATTATTAC	AGTACTGGTG	GCACTCAGC	''ጥጥሮር'' አለርጥ
	GATAGAGTCGCGA	AATAATAATG'	TCATGACCAC	CGTGAGTCG	AAGGTTCA
601	CTGGCTCGTTCCT	TTATAATTTG	CATCCAAATC	ATTTCAGAA	GCAGCAAG
	GACCGAGCAAGGA	AATATTAAAC	GTAGGTTTAC	TAAAGTCTT	'CGTCGTTC
C = -					
02I	ATTCCAATATATT	GAGGGAGAAA'	TGCGCACGAG	<b>SAATTAGGTA</b>	CAACCGGA
	TAAGGTTATATAA	CTCCCTCTTT	ACGCGTGCTC	TTAATCCAT	GTTGGCCT

# FIGURE 36C (P2)

- 701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
  AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTTCTTTGCTTATATTCCGGTCATGGGCTAATTTTAATGC AGCAGTGTCAAAAGAAAGGAATATAAGGCCAGTACCCGATTAAAATTACG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
  GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT

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### FIGURE 36C (P3)

- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
  CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP274

# FIGURE 36D

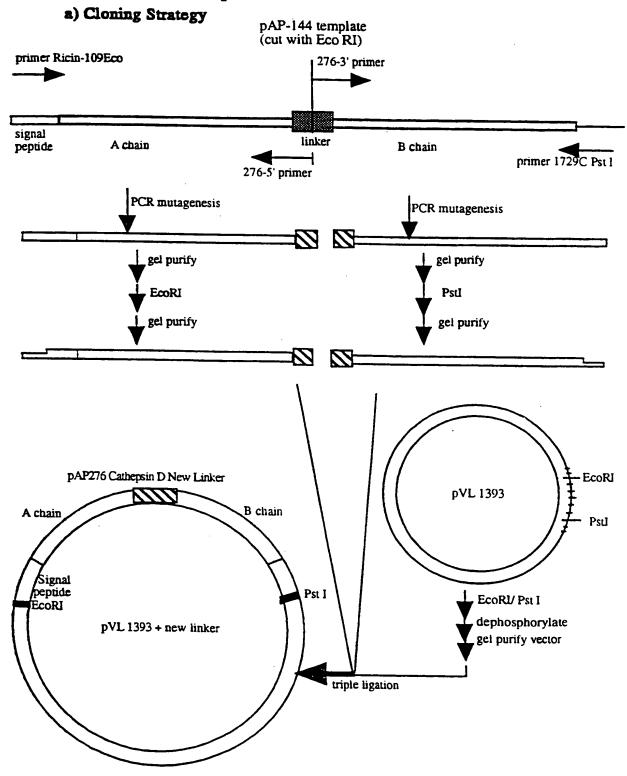
Amino acid sequence Comparison of Mutant Preproricin Linker region of Cathepsin L to Wild Type

Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-274 (Cathepsin L)linker: A chain- S L L I F R S W A N F N -B chain

### FIGURE 37A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# FIGURE 37B

# Sequence of Cathepsin D Linker Region

#### WT preprocin linker

TOTTTGCTTATAAGGCCA   GT	GTTATTGTTATCACCGCTGATGTTTGT -3' GGTACCAAATTTTAAT
3'-AGCAGTGTCAAAAGACCACAACAGTAGCGA -5 primer 276-5'	•
1) PCR muta <sub>i</sub>	genesis
2) Ligate with	1 pVL1393
pAP 276 link (Cathepsin DTCTGGTGTTGTCATCGCT   AC	Variant) TGTTATTGTTATCACC

### 171/254 FIGURE 37C (P1)

Sequence of pAP276 insert

				•		
	•	10	20	30 	40	50 I
1	GAATTCAT	GAAACCGGG!	AGGAAATACTA	TTGTAATAT	ו GGATGTATGC	ן 'אביד
	CTTAAGTA	CTTTGGCCC.	rcctttatga:	TAACATTATA	CCTACATACG	TCA
51	GGCAACAT	GCTTTGTT	TTGGATCCACC	TCAGGGTGG'	רייייייייירט כט ייי	T 3 C
	CCGTTGTA	CCGAAACAA	AACCTAGGTGG	SAGTCCCACC	AGAAAGTGTA	ATC
101	AGGATAAC	AACATATTC	CCCAAACAATA	CCCAATTAT	AAACTTTACC	ACA
	TCCTATTG!	ITGTATAAG(	GGTTTGTTAT	GGGTTAATA	ITTGAAATGG	TGT
151	GCGGGTGC	CACTGTGCA	AAGCTACACAA	ACTTTATCA	SAGCTGTTCC	CCC
	CGCCCACG	STGACACGT	TTCGATGTGTT	TGAAATAGT	CTCGACAAGC	CGG CCC
					•	
201	TCGTTTAA	CAACTGGAG	CTGATGTGAGA	CATGATATA	CAGTGTTGC	CAA
	AGCAAATT	STTGACCTC	GACTACACTCT	GTACTATAT	GTCACAACG	GTT
251	A CA CA COOR					
	TCTCTCNN	GITTGCCT?	TAAACCAACG	GTTTATTTT	AGTTGAACTC	TCA
	TOTCTCAM	CAAACGGA	TATTTGGTTGC	CAAATAAAA1	CAACTTGAG	AGT
301	AATCATGC	AGAGCTTTCT	rgttacattag	:೧೯೭೩ ಕ್ಷಾ	רכז ככז איינים:	3.003
	TTAGTACG:	rctcgaaagi	ACAATGTAATC	GCGACCTAC	AGTGGTTA CC	AIA Tat
351	TGTGGTCG	SCTACCGTG	CTGGAAATAGC	GCATATTTC	TTCATCCTG	ACA
	ACACCAGC	CGATGGCAC	GACCTTTATCG	CGTATAAAG	AAGTAGGAC	TGT
401						
101	TACTCCTT	SATGCAGAA(	CAATCACTCA	TCTTTTCACT	GATGTTCAA.	TAA
	INGICCII	LIACGICTI	CGTTAGTGAGT	'AGAAAAGTG <i>I</i>	ACTACAAGTT"	TTA
451	CGATATAC	ATTCGCCTTT	rggtggtaatt	יא יייני אייני אייני		
	GCTATATG	PAAGCGGAAZ	ACCACCATTAA	TACTATACAC ;	I GAACAAC'I"	rgc
				THCIMICIGA	MCTIGTTGA	ACG
501	TGGTAATC:	rgagagaaai	ATATCGAGTTG	GGAAATGGT	CACTAGAGG	N.C.C
	ACCATTAG	ACTCTCTTT	TATAGCTCAAC	CCTTTACCAC	GTGATCTCC	rcc Tcc
<b></b> -				•		
551	CTATCTCA	GCGCTTTAT	TATTACAGTAC	TGGTGGCACT	CAGCTTCCA	ACT
	GATAGAGT	CGCGAAATA	ATAATGTCATG	ACCACCGTG	AGTCGAAGGT	<b>IGA</b>
601	CTGGCTCG	PTCCTTTATE	AATTTGCATCC	יא א א א א א א א א א א א	<b>.</b>	_
	GACCGAGC	AAGGAAATAT	TTAAACGTAGG	·MARIGATTT !TTTN CTTN x x x	AGAAGCAGC	AAG
				TIACTAAA(	TCTTCGTCG	rtc

651 ATTCCAATATATTGAGGGAGAAATGCGCACGAGAATTAGGTACAACCGGA TAAGGTTATATAACTCCCTCTTTACGCGTGCTCTTAATCCATGTTGGCCT

### FIGURE 37C (P2)

701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT

- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- B01 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTTCTGGTGTTGTCATCGCTACTGTTATTGTTATCACCGC AGCAGTGTCAAAAGACCACAACAGTAGCGATGACAATAACAATAGTGGCG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
  GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCGGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
  AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT

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### FIGURE 37C (P3)

- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
  TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGÆTCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP276

#### FIGURE 37D

Amino acid sequence Comparison of Mutant Preproricin Linker region of Cathepsin D to Wild Type

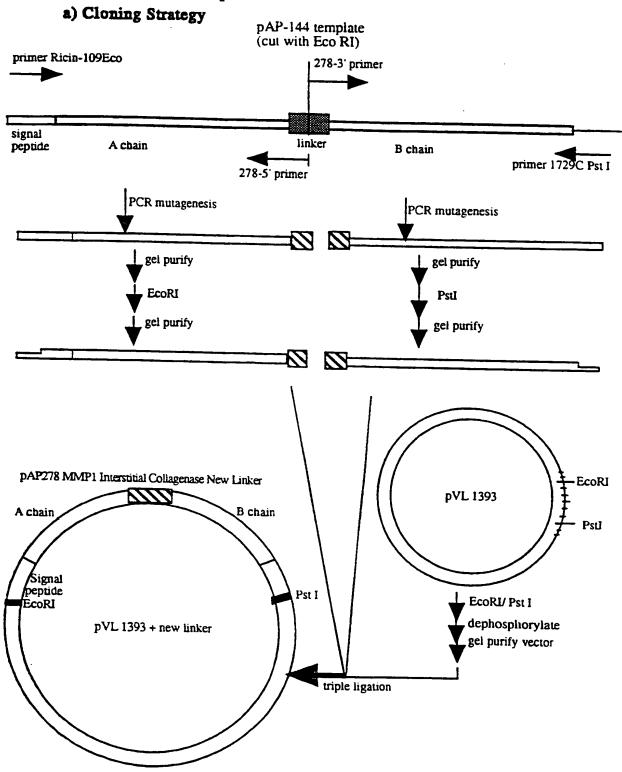
Wild type ricin linker: A chain- S L L I R P V V P N F N -B chain

pAP-276 (Cathepsin D) linker: A chain- S G V V I A T V I V I T -B chain

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### FIGURE 38A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



### FIGURE 38B

# Sequence of MMP-1 (Interstitial collagenase) Linker Region

#### WT preprocin linker

primer 278-3
5'- ATTTGGGGACAGTTTAATGCTGATGTTTGT -3'
TCTTTGCTTATAAGGCCA   GTGGTACCAAATTTTAAT
AGAAACGAATATTCCGGT CACCATGGTTTAAAATTA
3'-AGCAGTGTCAAAAGAAACCCAGGAGTTCCG -5' primer 278-5'
1) PCR mutagenesis
2) Ligate with pVL1393
pAP 278 linker
(MMP-1 variant)
TCTTTGGGTCCTCAAGGC ATTTGGGGACAGTTTAAT
AGAAACCCAGGAGTTCCG TAAACCCCTGTCAAATTA

# FIGURE 38C (P1)

Sequence of pAP278 insert

	10 	20	30	40	50
1	GAATTCATGAAACO	I CGGGAGGAAAT GCCCTCCTTT	ACTATTGTA TGATAACAT	 ATATGGATGTA IATACCTACAT	 TGCAGT ACGTCA
51	GGCAACATGGCTT	TGTTTTGGATO	CCACCTCAGG(	GTGGTCTTTCA	CATTAG
	CCGTTGTACCGAA	ACAAAACCTAO	GTGGAGTCC	CACCAGAAAGT	CGTAATC
101	AGGATAACAACAT	ATTCCCCAAA(	CAATACCCAA	PTATAAACTTT	ACCACA
	TCCTATTGTTGTA	TAAGGGGTTTC	STTATGGGTT	AATATTTGAA2	TGGTGT
151	GCGGGTGCCACTG	TGCAAAGCTAC	CACAAACTTT	ATCAGAGCTGT	TCGCGG
	CGCCCACGGTGAC	ACGTTTCGATC	GTGTTTGAAA	FAGTCTCGACA	AGCGCC
201	TCGTTTAACAACT(	GGAGCTGATG1	GAGACATGA:	TATACCAGTGT	TGCCAA
	AGCAAATTGTTGA	CCTCGACTACA	CTCTGTACT	ATATGGTCACA	ACGGTT
251	ACAGAGTTGGTTT(	GCCTATAAACC	CAACGGTTTA:	rttagttgaa	CTCTCA
	TGTCTCAACCAAA	CGGATATTTGC	STTGCCAAAT	Aaaatcaactt	GAGAGT
301	AATCATGCAGAGC TTAGTACGTCTCG	TTTCTGTTACA AAAGACAATGT	ATTAGCGCTG	GATGTCACCAA CTACAGTGGTT	TGCATA ACGTAT
351	TGTGGTCGGCTAC ACACCAGCCGATG	CGTGCTGGAA <i>I</i> GCACGACCTTT	ATAGCGCATA:	ITTCTTTCATC AAAGAAAGTAG	CTGACA GACTGT
401	ATCAGGAAGATGC	AGAAGCAATC <i>I</i>	CTCATCTTT	ICACTGATGTT	CAAAAT
	TAGTCCTTCTACG	TCTTCGTTAG1	CGAGTAGAAA	AGTGACTACAA	GTTTTA
451	CGATATACATTCG	CCTTTGGTGGT	TAATTATGAT.	AGACTTGAACA	ACTTGC
	GCTATATGTAAGC	GGAAACCACC	ATTAATACTA	ICTGAACTTGI	TGAACG
501	TGGTAATCTGAGA ACCATTAGACTCT	GAAAATATCG <i>I</i> CTTTTATAGC1	AGTTGGGAAA'	IGGTCCACTAG ACCAGGTGATC	AGGAGG TCCTCC
<b>5</b> 51	CTATCTCAGCGCT GATAGAGTCGCGA	TTATTATTAC! AATAATAATGI	AGTACTGGTG CATGACCAC	GCACTCAGCTT CGTGAGTCGA2	CCAACT
601	CTGGCTCGTTCCT	TTATAATTTG(	CATCCAAATG	ATTTCAGAAGC	CAGCAAG
	GACCGAGCAAGGA	AATATTAAAC(	GTAGGTTTAC	TAAAGTCTTCC	STCGTTC
<b>65</b> 1	ATTCCAATATATT TAAGGTTATATAA	GAGGGAGAAA: CTCCCTCTTI	rgcgcacgag Acgcgtgctc	AATTAGGTACA TTAATCCATGT	ACCGGA

### FIGURE 38C (P2)

- 701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGACTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTTCTTTGGGTCCTCAAGGCATTTGGGGACAGTTTAATGC AGCAGTGTCAAAAGAAACGCAGGAGTTCCGTAAACCCCTGTCAAATTACG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT

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#### FIGURE 38C (P3)

- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP278

### FIGURE 38D

Figure 38. d) Amino acid sequence Comparison of Mutant Preproricin Linker region of MMP-1 (Interstitial collagenase) to Wild Type

Wild type ricin linker:

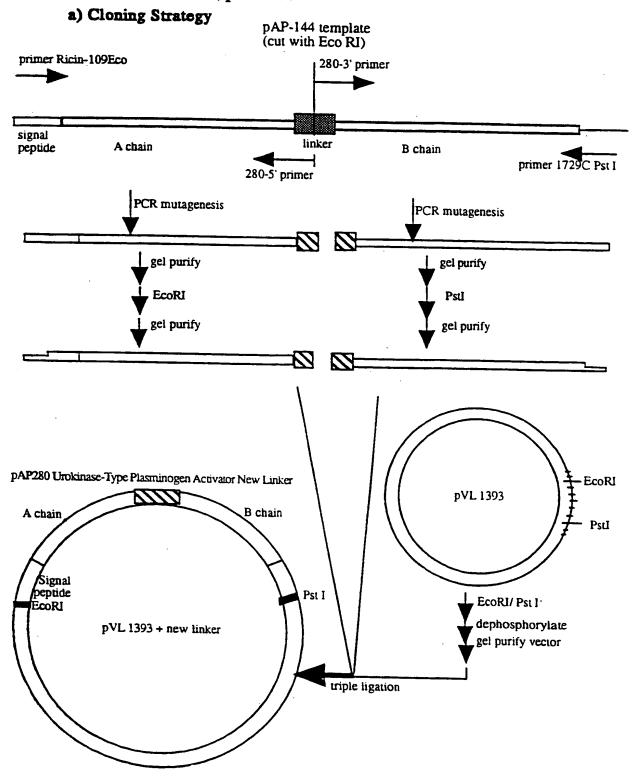
A chain- S L L I R P V V P N F N -B chain

pAP-278 (MMP-1) linker:

A chain- S L G P Q G I W G Q F N -B chain

#### FIGURE 39A

PCR Mutagenesis of Preprozicin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# FIGURE 39B

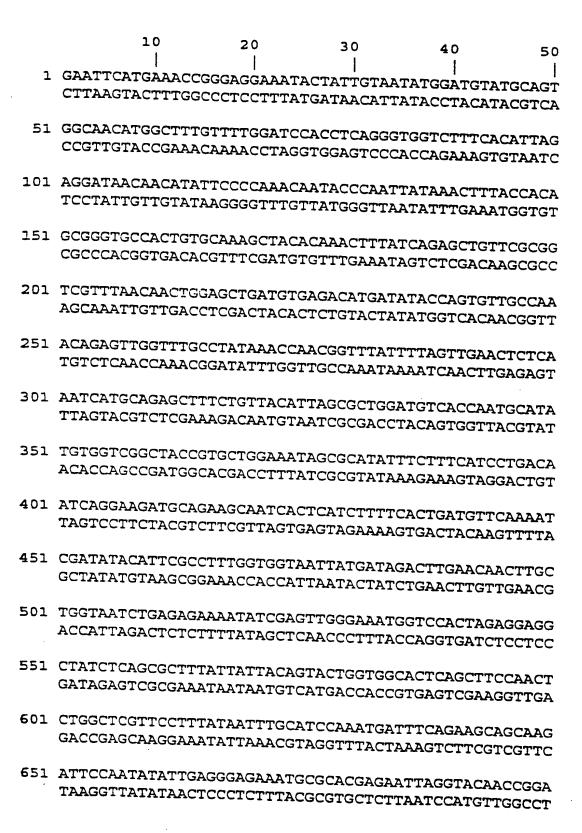
# Sequence of Urokinase-Type Plasminogen Activator Linker Region

#### WT preprocin linker

	Primer 280-3'
	5'- GTTGTCGGTGGCTCTGTAGCTGATGTTTGT -3'  TCTTTGCTTATAAGGCCA GTGGTACCAAATTTTAAT
	********* **
3'-AGCAGTGTCAAA	TTTTTTAGGGGACCTTCT -5'
I	Primer 280-5'
	1) PCR mutagenesis
	2) Ligate with pVL1393
A	pAP 280 linker (uPA variant) AAAAATCCCCTGGAAGA   GTTGTCGGTGGCTCTGTA
1	TTTTTAGGGGACCTTCT   CAACAGCCACCGAGACAT

### FIGURE 39C (P1)

Sequence of pAP280 insert



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# FIGURE 39C (P2)

701	GATCTGCACCACATCCTACCTACCTACCTACCTACCTACC
	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
	TATIGIGAACICTTATCAACCCCCTCT

- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- B51 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTAAAAAATCCCCTGGAAGAGTTGTCGGTGGCTCTGTAGC AGCAGTGTCAAATTTTTTAGGGGACCTTCTCAACAGCCACCGAGACATCG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT

#### FIGURE 39C (P3)

- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
  CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP280

# FIGURE 39D

Figure 39. d) Amino acid sequence Comparison of Mutant Preproricin Linker region of Urokinase-Type Plasminogen Activator to Wild Type

Wild type ricin linker:

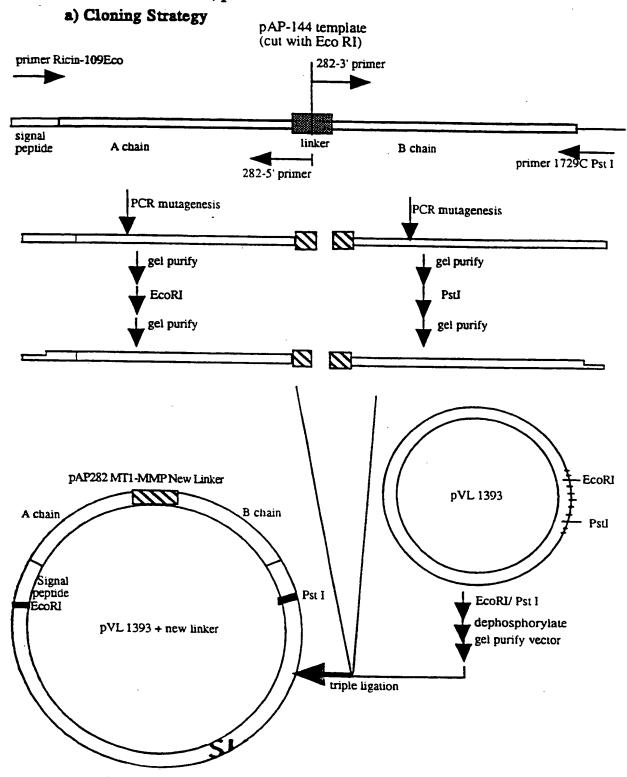
A chain- S L L I R P V V P N F N -B chain

pAP-280 (uPA) linker:

A chain- K K S P G R V V G G S V-B chain

#### FIGURE 40A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



### FIGURE 40B

# Sequence of MT-MMP Linker Region

### WT preprocin linker

TCTTTGCTTATAAGGCCA LC	TCCTGGTATTCTTGGCGCTGATGTTTGT: -3'  FGGTACCAAATTTTAAT
1) PCR mura	genesis
2) Ligate with	a pVL1393
pAP 282 link (MT-MMP v 	rariant)

### FIGURE 40C (P1)

Sequence of pAP282 insert

		10 	20 1	30 I	40	50
ı	GAATTCAT	GAAACCGG	GAGGAAAT	' ACTATTGI	I AATATGGAT	  GTATGCAGT
	CTTAAGTA	CTTTGGCC	CTCCTTTA	TGATAACA	TTATACCTA	CATACGTCA
51	GGCAACAT	GGCTTTGT	TTTGGAT	CACCTCAC	GGTGGTCTT	TCACATTAG
						AGTGTAATC
101	AGGATAAC	AACATATT	CCCCAAAC	AATACCC	AATTATAAA	TTTACCACA
						SAAATGGTGT
151						TGTTCGCGG
						SACAAGCGCC
201						STGTTGCCAA
						CACAACGGTT
251	ACAGAGTT	GGTTTGCC	TATAAACO	AACGGTTI	TATTTTAGTT	GAACTCTCA
						ACTTGAGAGT
301	AATCATGO	AGAGCTTT	CTGTTAC	ATTAGCGCT	rggatgtcac	CCAATGCATA
						GGTTACGTAT
351	TGTGGTCG	GCTACCGT	GCTGGAA	ATAGCGCA:	PATTTCŢTT(	CATCCTGACA
	•.					STAGGACTGT
401	ATCAGGAA	GATGCAGA	AGCAATC	ACTCATCT	TTTCACTGAT	TGTTCAAAAT
						ACAAGTTTTA
451	CGATATAC	CATTCGCCT	TTGGTGG:	PAATTATG	ATAGACTTG	AACAACTTGC
						TTGTTGAACG
501	TGGTAATO	TGAGAGAA	AATATCG	AGTTGGGAI	AATGGTCCA	CTAGAGGAGG
					,	GATCTCCTCC
551	CTATCTCA	AGCGCTTTA	TTATTAC	AGTACTGG'	IGGCACTCA	GCTTCCAACT
						CGAAGGTTGA
601	CTGGCTC	STTCCTTTA	TAATTTG	CATCCAAA'	TGATTTCAG	AAGCAGCAAG
_						TTCGTCGTTC
651	ATTCCAA'	TATATTGAC	GGAGAAA	TGCGCACG	AGAATTAGG:	TACAACCGGA
	TAAGGTT	ATATAACT	ECCTCTTT.	ACGCGTGC'	TCTTAATCC	ATGTTGGCCT

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# FIGURE 40C (P2)

701	GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA
	CTAGACGTCGTCTACCATCCCATCCCATCCCATCCCATC
	CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT

- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
  AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTCCCCAAGGACTCCTAGGGGCTCCTGGTATTCTTGGCGC AGCAGTGTCAAAGGGGTTCCTGAGGATCCCCGAGGACCATAAGAACCGCG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT
  GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT

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# FIGURE 40C (P3)

- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP282

# FIGURE 40D

Amino acid sequence Comparison of Mutant Preproricin Linker region of MT-MMP to Wild Type

Wild type ricin linker:

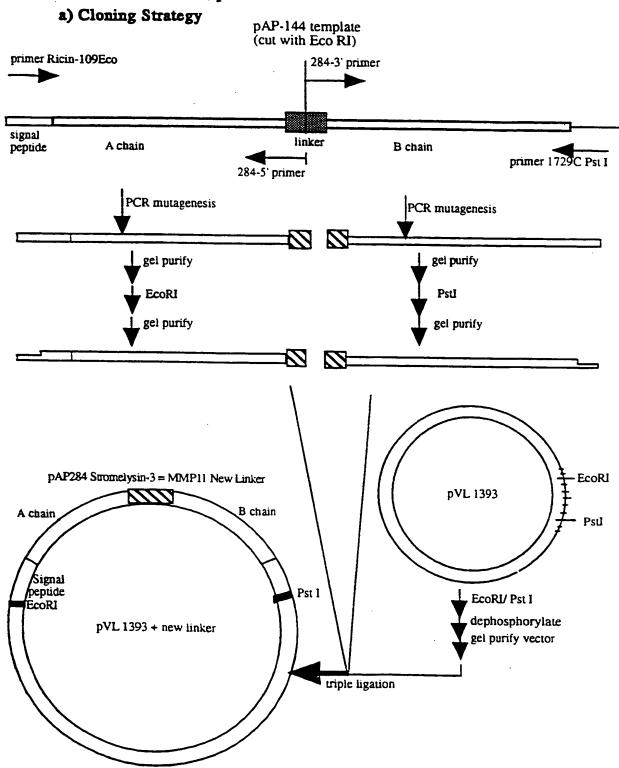
A chain- S L L I R P V V P N F N -B chain

pAP-282 (MT-MMP) linker:

A chain- P Q G L L G A P G I L G-B chain

## FIGURE 41A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# Sequence of MMP-11 (Stromelysin-3) Linker Region

WT preprocin linker

primer 284-3

5 - ATGGGAAGAGGCCATGCTTTAGTTCATGTCGAAGAGCCTCACACTGCTGATGTTTGTATGGAT-3

----AGNAACGAATATTCCGGT | CACCATGGTTTAAAATTA----

3. GGTGGTAGCAGTGTCAAAGTGCCGGGGCTCCCAAATTCTCACCCTAAAATACTTAGACTGCAG -5'

primer 284-5'

1) PCR mutagenesis

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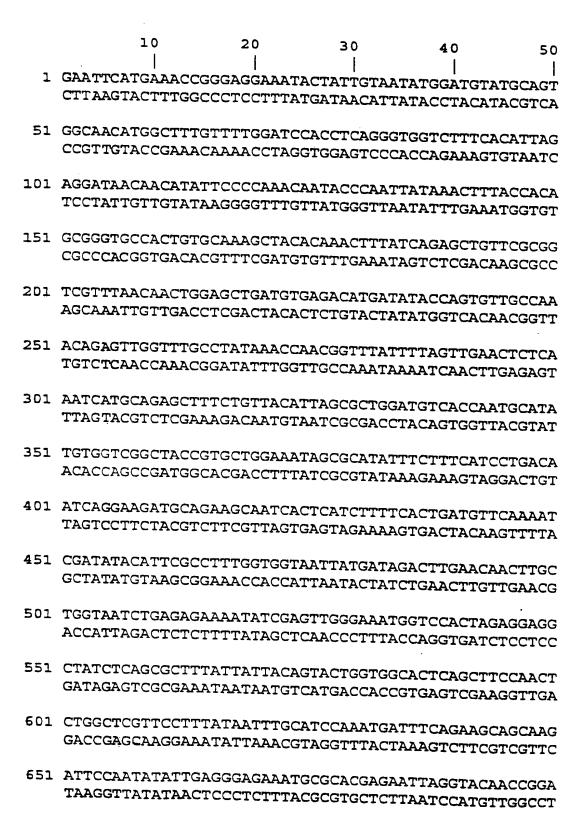
2) Ligate with pVL 1393

(MMP-11 variant) pAP 284 linker

---CACGGCCCCGAGGGTTTAAGAGTGGGATTTTATGAATCTGACGTC|ATGGGÁAGAGGCCATGCTCGTTTAGTTCATGTGAAGAGCCTCACACT------GTGCCGGGGCTCCCAAATTCTCACCCTAAAATACTTAGACTGCAG|TACCCTTCTCCGGTACGAGCAAATCAAGTACAGCAACTCGGAGTGTGA---

# FIGURE 41C (P1)

Sequence of pAP284 insert



# 196/254 FIGURE 41C (P2)

- 701 GATCTGCACCAGATCCTAGCGTAATTACACTTGAGAATAGTTGGGGGAGA CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTT AGCAGTGTCAAA

Linker Sequence:

CACGGCCCCGAGGGTTTAAGAGTGGGATTTTATGAATCTGACGTCATGGG GTGCCGGGGCTCCCAAATTCTCACCCTAAAATACTTAGACTGCAGTACCC

AAGAGGCCATGCTCGTTTAGTTCATGTCGAAGAGCCTCACACT TTCTCCGGTACGAGCAAATCAAGTACAGCAACTCGGAGTGTGA

949 GC CG

- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG

# FIGURE 41C (P3)

- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
  AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
  TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA
- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
  CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

# FIGURE 41D

Amino acid sequence Comparison of Mutant Preproricin Linker region of MMP-11 (Stromelysin-3) to Wild Type

Wild type ricin linker:

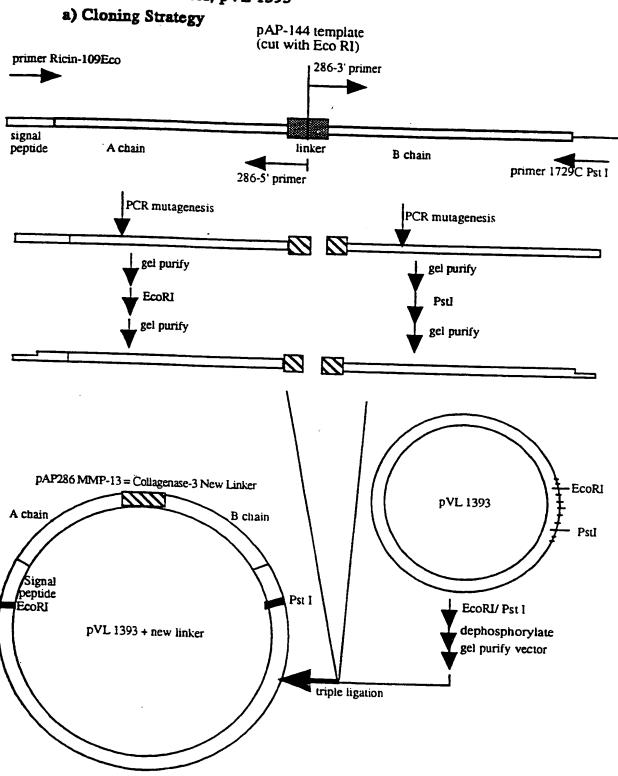
A chain- S L L I R P V V P N F N -B chain

pAP-284 (MMP-11) linker:

A chain- H G P E G L R V G F Y E S D V M G R G H A R L V H V E E P H T -B chain

# FIGURE 42A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



----

# FIGURE 42B

# Sequence of MMP-13 = Collagenase-3 Linker Region

## WT preprocin linker

primer 286-3' 5'- GGTCAACGAGGCATTGTCGC	
TCTTTGCTTATAAGGCCA   GTGGTACCAAATTTTAAT-AGAAACGAATATTCCGGT   CACCATGGTTTAAAATTA-	
3'-AGCAGTGTCAAACCTGGAGTCCCCGAACGA -5' primer 286-5'	
1) PCR mutagenesis	
2) Ligate with pVL1393	
pAP 286 linker (MMP-13 variant)	•
GGACCTCAGGGGCTTGCT   GGTCAACGAGGCATTGTC	

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# FIGURE 42C (P1)

### Sequence of pAP286 insert

	10	20	30	40	50
1	GAATTCATGAAACCG CTTAAGTACTTTGGC	I GGAGGAAA: CCTCCTTT	, , PACTATTGTAA ATGATAACATT	  TATGGATGTA  ATACCTACAT	I TGCAGT ACGTCA
51	GGCAACATGGCTTTG	TTTTGGAT(	CCACCTCAGG	TGGTCTTTCA	CATTAG
	CCGTTGTACCGAAAC	AAAACCTA(	GGTGGAGTCC	CACCAGAAAGT	GTAATC
101	AGGATAACAACATAT	TCCCCAAA(	CAATACCCAAT	TATAAACTTT	ACCACA
	TCCTATTGTTGTATA	AGGGGTTT(	GTTATGGGTT <i>I</i>	ATATTTGAAA	TGGTGT
151	GCGGGTGCCACTGTG	CAAAGCTA	CACAAACTTTA	ATCAGAGCTGT	TCGCGG
	CGCCCACGGTGACAC	GTTTCGAT	GTGTTTGAAA1	FAGTCTCGACA	AGCGCC
201	TCGTTTAACAACTGG	AGCTGATG	TGAGACATGA1	IATACCAGTGT	TGCCAA
	AGCAAATTGTTGACC	TCGACTAC	ACTCTGTACTA	ATATGGTCACA	ACGGTT
251	ACAGAGTTGGTTTGC	CTATAAAC	CAACGGTTTA1	AADTTDATTT1	CTCTCA
	TGTCTCAACCAAACG	GATATTTG	GTTGCCAAATA	TTDAADTAAA	GAGAGT
301	AATCATGCAGAGCTT	TCTGTTAC	ATTAGCGCTG(	SATGTCACCAA	TGCATA
	TTAGTACGTCTCGAA	AGACAATG	TAATCGCGAC(	STACAGTGGTT	ACGTAT
351	TGTGGTCGGCTACCG	TGCTGGAA	ATAGCGCATAT	ITTCTTTCATC	CTGACA
	ACACCAGCCGATGGC	ACGACCTT	TATCGCGTATA	AAAGAAAGTAG	GACTGT
401	ATCAGGAAGATGCAG	SAAGCAATC	ACTCATCTTT	CACTGATGTT	CAAAAT
	TAGTCCTTCTACGTC	CTTCGTTAG	TGAGTAGAAA	AGTGACTACAA	GTTTTA
451	CGATATACATTCGCC	TTTGGTGG	TAATTATGAT <i>I</i>	AGACTTGAACA	ACTTGC
	GCTATATGTAAGCGC	SAAACCACC	ATTAATACTA:	ICTGAACTTGT	TGAACG
501	TGGTAATCTGAGAGA	AAATATCG	AGTTGGGAAA:	IGGTCCACTAG	AGGAGG
	ACCATTAGACTCTCT	TTTATAGC	TCAACCCTTT	ACCAGGTGATC	TCCTCC
551	CTATCTCAGCGCTTT	CATTATTAC	AGTACTGGTG(	GCACTCAGCTT	CCAACT
	GATAGAGTCGCGAA	ATAATAATG	TCATGACCAC(	CGTGAGTCGAA	GGTTGA
601	CTGGCTCGTTCCTTT	TATAATTTG	CATCCAAATG	ATTTCAGAAGC	AGCAAG
	GACCGAGCAAGGAA	ATATTAAAC	GTAGGTTTAC	TAAAGTCTTCG	TCGTTC
651	ATTCCAATATATTGA	AGGGAGAAA	TGCGCACGAG	AATTAGGTACA	ACCGGA
	TAAGGTTATATAAC	ICCCTCTTT	ACGCGTGCTC	TTAATCCATGI	TGGCCT
701	GATCTGCACCAGAT	CCTAGCGTA	ATTACACTTG	AGAATAGTTGG	GGGAGA

# FIGURE 42C (P2)

- 751 CTTŢCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTGGACCTCAGGGGCTTGCTGGTCAACGAGGCATTGTCGC AGCAGTGCAAACCTGGAGTCCCCGAACGACCAGTTGCTCCGTAACAGCG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
  TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

### FIGURE 42C (P3)

### GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC
  CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

# FIGURE 42D

Amino acid sequence Comparison of Mutant Preproricin Linker region of MMP-13 (Collagenase-3) to Wild Type

Wild type ricin linker:

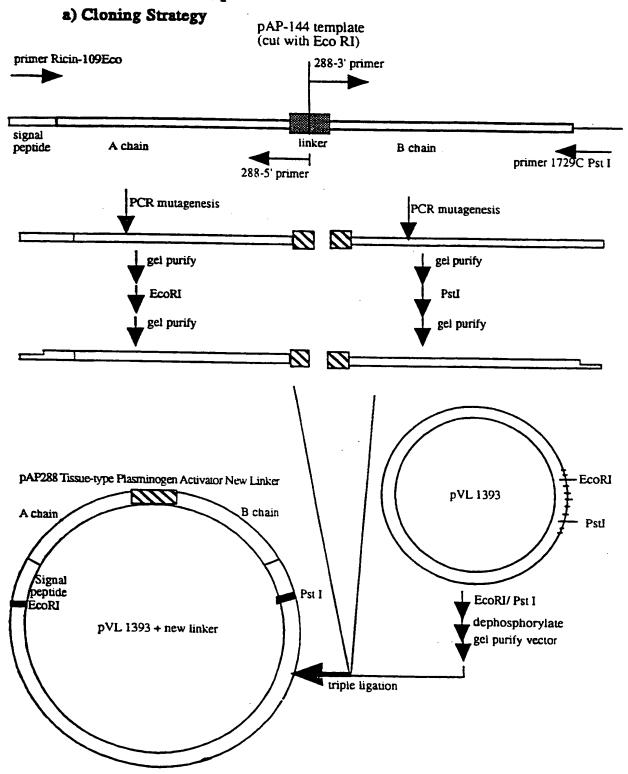
A chain- S L L I R P V V P N F N -B chain

pAP-286 (MMP-13) linker:

A chain- G P Q G L A G Q R G I V -B chain

# FIGURE 43A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# FIGURE 43B

# Sequence of Tissue-type Plasminogen Activator (tPA) Linker Region

### WT preprocin linker

	primer 5'-	288-3' GGTCGTAAAGCTCTTGAAGC	TGATGTTTGT -3'
TC7	TTTGCTTATAAGGCCA AAACGAATATTCCGGT	GTGGTACCAAATTTTAAT   CACCATGGTTTAAAATTA	
3'-AGCAGTGTCAAACCC	GCCTAGACCCGTTTCC Lmer 288-5'	-5'	
	1) PCR n	nutagenesis	
	2) Ligate	with pVL1393	
	pAP 288 (tPA var	· · · · · ·	
GC	<b>ECGGATCTGGGCAAAG</b>	imit) G GGTCGTAAAGCTCTTGAA C CCAGCATTTCGAGAACTT	

# FIGURE 43C (P1)

Sequence of pAP288 insert

	10	:	20	30	40	50
1	GAATTCATGA CTTAAGTACT	AACCGGGA( TTGGCCCT(	GAAATACTA CCTTTATGA:	I ATTGTAATAT AACATTATA	i GGATGTATGC <i>i</i> CCTACATACG1	IGT CA
51	GGCAACATGG CCGTTGTACC	CTTTGTTT: GAAACAAA	IGGATCCAC( ACCTAGGTG(	CTCAGGGTGG GAGTCCCACC	TCTTTCACATT AGAAAGTGTA	îag Atc
101	AGGATAACAA TCCTATTGTT	CATATTCC( GTATAAGG(	CCAAACAATI GGTTTGTTA:	ACCCAATTAT. IGGGTTAATA	AAACTTTACC <i>I</i> TTTGAAATGGT	ACA IGT
151	GCGGGTGCCA CGCCCACGGT	CTGTGCAA GACACGTT	AGCTACACAI ICGATGTGT:	ACTTTATCA TTGAAATAGT	GAGCTGTTCGC CTCGACAAGCG	GG GCC
201	TCGTTTAACA AGCAAATTGT	ACTGGAGC' TGACCTCG	IGATGTGAGA ACTACACTC	ACATGATATA IGTACTATAT	CCAGTGTTGCC GGTCACAACGO	LAA STT
251	ACAGAGTTGG TGTCTCAACC	TTTGCCTA: AAACGGATI	TAAACCAAC( ATTTGGTTG(	GTTTATTTT CAAATAAAA	AGTTGAACTCT TCAACTTGAGA	'CA \GT
301	AATCATGCAG	AGCTTTCT	GTTACATTAC	GCGCTGGATG	TCACCAATGCA AGTGGTTACGT	
351	TGTGGTCGGC	TACCGTGC	IGGAAATAGO	ገርር ልጥል ጥጥጥ <i>ር</i> ነ	TTTCATCCTGA AAAGTAGGACT	
401	ATCAGGAAGA	TGCAGAAG	CAATCACTC	<u>ዓ</u> ጥርጥጥጥር አርነ	TGATGTTCAAA ACTACAAGTTI	\ <b>7</b> \ <b>6</b> \
451	CGATATACAT	TCGCCTTT	GGTGGTAAT	ГАТСАТАСАС	TTGAACAACTT AACTTGTTGAA	
501	TGGTAATCTG	AGAGAAAA'	TATCGAGTT	GGAAATGGT	CCACTAGAGGA GGTGATCTCCT	
551	CTATCTCAGC	GCTTTATT	ATTACAGTAC	Treeteca	TCAGCTTCCAA AGTCGAAGGTT	
601	CIGGCICGII	CCTTTATA	ATTTGCATC	<sup>-</sup>	CAGAAGCAGCA GTCTTCGTCGT	
651	ATTCCAATAT	ATTGAGGG	AGAAATGCGG	~ ね	AGGTACAACCG TCCATGTTGGC	
701					TAGTTGGGGG	

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# FIGURE 43C (P2)

CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
--

751	
, 51	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT
	CAAA CCTCA CCTTTA A CTTTTA A C
	GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCACCTTA

- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
  AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTGGCGGATCTGGGCAAAGGGGTCGTAAAGCTCTTGAAGC AGCAGTGTCAAACCGCCTAGACCCGTTTCCCCAGCATTTCGAGAACTTCG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
  TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

# FIGURE 43C (P3)

GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP288

# FIGURE 43D

Amino acid sequence Comparison of Mutant Preproricin Linker region of Tissue-type Plasminogen Activator (tPA) to Wild Type

Wild type ricin linker:

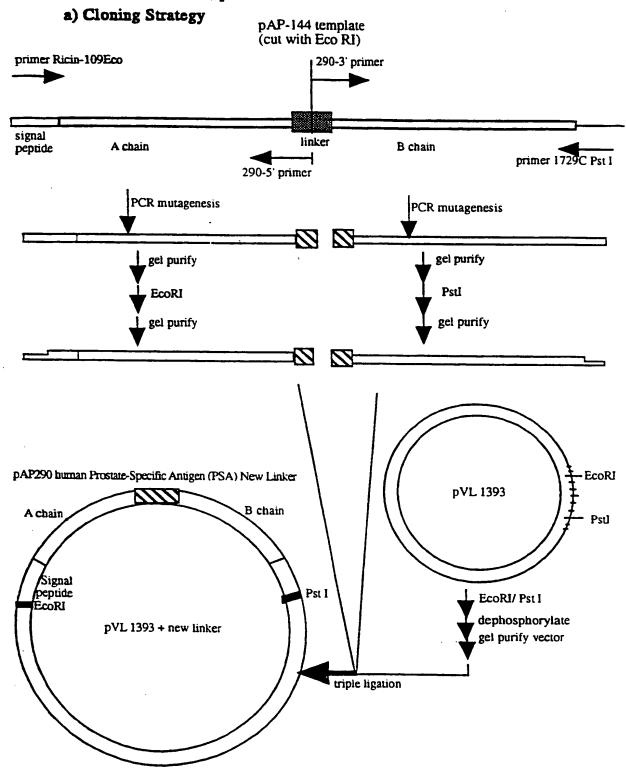
A chain- S L L I R P V V P N F N -B chain

pAP-288 (tPA) linker:

A chain- G G S G Q R G R K A L E-B chain

### FIGURE 44A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# FIGURE 44B

# Sequence of human Prostate-Specific Antigen (PSA) Linker Region

## WT preprocin linker

primer 290-3' 5'- TCTTCCGATATTTTTAATGCTGATGTTTGT -3'
TCTTTGCTTATAAGGCCA   GTGGTACCAAATTTTAATAGAAACGAATATTCCGGT   CACCATGGTTTAAAATTA
3'-AGCAGTGTCAAAAGAACAGTCGAGAAGAG -5' primer 290-5'
1) PCR mutagenesis 2) Ligate with pVL1393
pAP 290 linker  (PSA variant) TCTTTGTCAGCTCTTCTC   TCTTCCGATATTTTTAATAGAAACAGTCGAGAAGAG   AGAAGGCTATAAAAATTA

# FIGURE 44C (P1)

### Sequence of pAP290 insert

		10	•	20		30	40	5	0
1	GAATTCA	TGAA <i>I</i>	CCGG	ا SAGGAAF	TACTA	। TTGTAA	ا TATGGAT	GTATGCAG	 T
	CTTAAGT.	ACTTI	GGCC	CTCCTTI	ATGAT.	AACATT.	ATACCTA	CATACGTC	A
51	GGCAACA	TGGCT	TTGT	TTGGAT	CCACC	TCAGGG	TGGTCTT	TCACATTA	G
	CCGITGI	ACCGA	AAACAA	AAACCTA	AGGTGG	AGTCCC	ACCAGAA	AGTGTAAT	С
101	AGGATAA	CAAC	TATTO	CCCAAA	CAATA	CCCAAT	TATAAAC	TTTACCAC	Α
								AAATGGTG	
151	GCGGGTG	CCACT	rgrgci	AAAGCTA	CACAA	ACTTTA	TCAGAGC	TGTTCGCG ACAAGCGC	G
201	TCGTTTA	ACAA	TGGA	SCTGATO	STGAGA	CATGAT	ATACCAG	TGTTGCCA	A
	AGCAAAT	TGTT	ACCT	CGACTA	CACTCT	GTACTA	TATGGTC	ACAACGGT	T
251	ACAGAGT	TGGT	rtgcc:	CAAATAT	CCAACG	GTTTAT	TTTAGTT	GAACTCTC	Α
	TGTCTCA	ACCAI	AACGG2	ATATTTO	GTTGC	CAAATA	AAATCAA	CTTGAGAG	T
301	AATCATG	CAGA	SCTTT	CTGTTA	CATTAG	CGCTGG	ATGTCAC	CAATGCAT	Α
	TTAGTAC	GTCT	CGAAA	GACAAT	STAATO	GCGACC	TACAGTG	GTTACGTA	T.
351	TGTGGTC	GGCT	ACCGT	GCTGGA	AATAGO	GCATAT	יייייטיייר.	ATCCTGAC	Δ.
	ACACCAG	CCGA!	IGGCA	CGACCT!	TATCO	CGTATA	AAGAAAG	TAGGACTG	T
401	ATCAGGA	AGAT	GCAGA	AGCAAT	CACTCA	TCTTTT	CACTGAT	GTTCAAAA	'n
	TAGTCCT	TCTA	CGTCT	TCGTTA	GTGAGI	'AGAAAA	GTGACTA	CAAGTTTT	Ά
451	CGATATA	CATT	CGCCT	TTGGTG	STAATI	ATGATA	GACTTGA	ACAACTTO	
	GCTATAI	GTAA	GCGGA	AACCAC	CATTA	TACTAT	CTGAACT	TGTTGAAC	:G
501	TGGTAAI	CTGA	GAGAA	AATATC	GAGTTO	GGAAAT	GGTCCAC	TAGAGGAG	;G
	ACCATTA	GACT	CTCTT	TTATAG	CTCAAC	CCTTTA	CCAGGTG	ATCTCCTC	:C
551	CTATCTO	AGCG	CTTTA	TTATTA	CAGTAC	TGGTGG	CACTCAG	CTTCCAAC	٠,
	GATAGAG	TCGC	GAAAT.	TAATAA	GTCATO	SACCACO	GTGAGTC	GAAGGTT	įΑ
601	CTGGCTC	CGTTC	CTTTA	TAATTT	GCATCO	CAAATGA	עדדרבב	AGCAGCA	٠.
	GACCGAC	CAAG	GAAAT	ATTAAA	CGTAGO	STTTACT	AAAGTCI	TCGTCGT	C:C
651	ATTCCA	ATATA	TTGAG	GGAGAA	ATGCGC	TACGAGE	<u>, аттасст</u>	ACAACCG	~ 7\
	TAAGGT	TATAT	AACTO	CCTCTT	TACGC	STGCTCT	TAATCC	TGTTGGC	T
701	GATCTG	CACCA	GATCC	TAGCGT	AATTAG	CACTTGA	AGAATAGT	TGGGGGA	3A

### FIGURE 44C (P2)

CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT

- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTTCTTTGTCAGCTCTTCTCTCTCTCCGATATTTTTAATGC AGCAGTGTCAAAAGAAACAGTCGAGAAGAGAGAGAGGCTATAAAAATTACG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATACAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTTGTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA
  CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
  TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

# FIGURE 44C (P3)

GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCACACCCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP290

# FIGURE 44D

Amino acid sequence Comparison of Mutant Preproricin Linker region of human Prostate-Specific Antigen (PSA) to Wild Type

Wild type ricin linker:

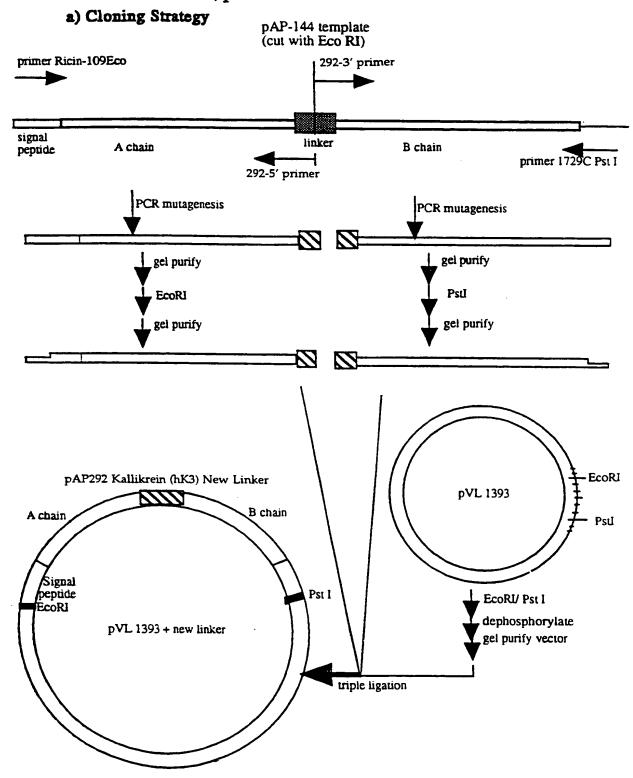
A chain- S L L I R P V V P N F N -B chain

pAP-290 (PSA) linker:

A chain- S L S A L L S S D I F N -B chain

### FIGURE 45A

PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



# FIGURE 45B

Sequence of Kallikrein (hK3) Linker Region

WT preprocin linker

primer 292-3' 5'- ATTATCGGTGGCTTTAATGCTGATGTTTGT -3'  * ** *******	
1) PCR mutagenesis	
2) Ligate with pVL1393	
pAP 292 linker	
(Kallikrein variant) ————————————————————————————————————	

# FIGURE 45C (P1)

### Sequence of pAP292 insert

	10	20	30	40	50
	1	1	1	. 1	1
1	GAATTCATGAAACCG	GGAGGAAI	<b>LTACTATTGTA</b>	ATATGGATGTA	LTGCAGT
	CTTAAGTACTTTGGC	CCTCCTT	ATGATAACAT!	<b>TATACCTACAT</b>	CACGTCA
51	GGCAACATGGCTTTG	TTTTGGA:	CCACCTCAGG	STGGTCTTC	CATTAG
	CCGTTGTACCGAAAC				
101	<b>AGGATAACAACATA</b>	TCCCCAA	ACARTACCCAA	TTATAAACTT:	TACCACA
	TCCTATTGTTGTATA	AGGGGTT'	IGTTATGGGTT.	AATATTTGAA	ATGGTGT
151	GCGGGTGCCACTGTG	CAAAGCT	ACACAAACTTT.	ATCAGAGCTG'	TTCGCGG
	CGCCCACGGTGACAC				
201	TCGTTTAACAACTG	AGCTGAT	GTGAGACATGA	TATACCAGTG'	TTGCCAA
	AGCARATTGTTGAC				
251	ACAGAGTTGGTTTG	CTATAAA	CCAACGGTTTA	TTTTAGTTGA	ACTCTCA
	TGTCTCAACCAAAC	GATATTT	GGTTGCCAAAT	AAAATCAACT'	TGAGAGT
301	AATCATGCAGAGCT:	TTCTGTTA	CATTAGCGCTG	GATGTCACCA	ATGCATA
	TTAGTACGTCTCGA	AAGACAAT	GTAATCGCGAC	CTACAGTGGT	TACGTAT
351	TGTGGTCGGCTACC	GTGCTGGA	AATAGCGCATA	TTTCTTTCAT	CCTGACA
	ACACCAGCCGATGG	CACGACCT	TTATCGCGTAT	'AAAGAAAGTA	GGACTGT
401	ATCAGGAAGATGCA	GAAGCAAT	CACTCATCTTT	TCACTGATGT	TCABBAT
	TAGTCCTTCTACGT	CTTCGTTA	GTGAGTAGAAA	AGTGACTACA	AGTTTTA
451	CGATATACATTCGC	CTTTGGTG	GTAATTATGAT	TAGACTTGAAC	AACTTGC
	GCTATATGTAAGCG	GAAACCAC	CATTAATACTA	TCTGAACTTG	TTGAACG
501	TGGTAATCTGAGAG	AAAATATO	GAGTTGGGAA	ATGGTCCACTA	GAGGAGG
	ACCATTAGACTCTC	TTTTATAC	CTCAACCCTT	TACCAGGTGAT	CTCCTCC
551	CTATCTCAGCGCTT	TATTATTA	CAGTACTGGT	GCACTCAGCT	TCCAACT
	GATAGAGTCGCGAA	ATAATAAT	GTCATGACCA	CCGTGAGTCGA	AGGTTGA
601	CTGGCTCGTTCCTT	TATAATT	GCATCCAAAT	GATTTCAGAAC	CAGCAAG
	GACCGAGCAAGGAA	ATATTAA	CGTAGGTTTA	CTARAGTCTTC	GTCGTTC
651	ATTCCAATATATTC	AGGGAGAI	ATGCGCACGA	GAATTAGGTAG	AACCGGA
	TAAGGTTATATAAC	TCCCTCT:	TACGCGTGCT	CTTAATCCATC	STIGGCCT
701	GATCTGCACCAGAT	CCTAGCG:	PARTTACACTT	GAGAATAGTT(	GGGGAGA

# FIGURE 45C (P2)

CTAGACGTGGTCTAG	SATCGCATTAATGTGAACTCTTATCAACCCCCT	.~~
~		יווייקיי

- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
  AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 901 TCGTCACAGTTTTCTTTGCCTAGATTTAAAATTATCGGTGGCTTTAATGC AGCAGTGTCAAAAGAAACGGATCTAAATTTTAATAGCCACCGAAATTACG
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT
  AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

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### FIGURE 45C (P3)

### GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP292

# FIGURE 45D

Amino acid sequence Comparison of Mutant Preproricin Linker region of Kallikrein (hK3) to Wild Type

Wild type ricin linker:

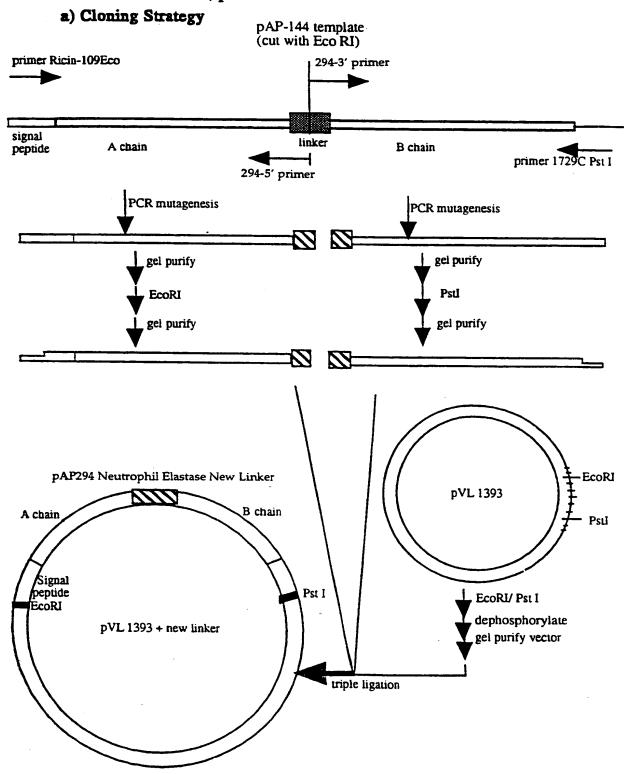
A chain- S L L I R P V V P N F N -B chain

pAP-292 (hK3) linker:

A chain- S L P R F K I I G G F N -B chain

# FIGURE 46A

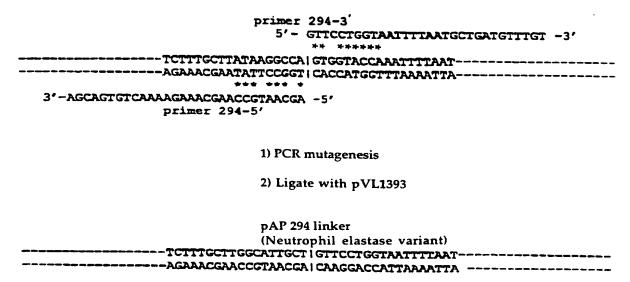
PCR Mutagenesis of Preproricin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



### FIGURE 46B

## Sequence of Neutrophil Elastase Linker Region

### WT preprocin linker



# FIGURE 46C (P1)

## Sequence of pAP294 insert

	10	20	30	40	50
1	GAATTCATGAAACCG CTTAAGTACTTTGGC	GGAGGAAA? CCTCCTTTI	IACTATTGTAI ATGATAACAT	 ATATGGATGT <i>I</i> FATACCTACAT	LTGCAGT CACGTCA
51	GGCAACATGGCTTTC	STTTTGGAT(	CCACCTCAGG(	STGGTCTTTC!	ACATTAG
	CCGTTGTACCGAAAC	CAAAACCTA(	GGTGGAGTCC(	CACCAGAAAG	IGTAATC
101	AGGATAACAACATAT	TCCCCAAA(	CAATACCCAA!	TTATAAACTT:	raccaca
	TCCTATTGTTGTATA	AAGGGGTTT	STTATGGGTTI	AATATTTGAA	Atggtgt
151	GCGGGTGCCACTGTG	SCAAAGCTA(	CACAAACTTT	atcagagetg:	TCGCGG
	CGCCCACGGTGACAC	SGTTTCGAT(	GTGTTTGAAA	Iagtetegaci	AAGCGCC
201	TCGTTTAACAACTGG	EAGCTGATG:	igagacatga:	TATACCAGTG:	TTGCCAA
	AGCAAATTGTTGACG	ETCGACTAC	Actetgtacti	ATATGGTCAC	AACGGTT
251	ACAGAGTTGGTTTGC	CTATAAAC	CAACGGTTTA:	TTTTAGTTGAI	actetea
	TGTCTCAACCAAACC	GATATTTG	GTTGCCAAAT	AAAATCAACT:	Igagagt
301	AATCATGCAGAGCTT	TTCTGTTAC:	ATTAGCGCTG	GATGTCACCAI	ATGCATA
	TTAGTACGTCTCGA	AAGACAATG	FAATCGCGAC	CTACAGTGGT	PACGTAT
351	TGTGGTCGGCTACCO	STGCTGGAA	ATAGCGCATA	TTTCTTTCAT(	CCTGACA
	ACACCAGCCGATGGO	CACGACCTT	IATCGCGTAT	AAAGAAAGTA(	SGACTGT
401	ATCAGGAAGATGCAG	Gaagcaatc	ACTCATCTTT	TCACTGATGT:	ICAAAAT
	TAGTCCTTCTACGTG	Cttcgttag	TGAGTAGAAA	AGTGACTACAI	AGTTTTA
451	CGATATACATTCGCC	CTTTGGTGG	TAATTATGAT.	AGACTTGAAC	AACTTGC
	GCTATATGTAAGCGC	GAAACCACC	ATTAATACTA	TCTGAACTTG	FTGAACG
501	TGGTAATCTGAGAG	AAAATATCG	AGTTGGGAAA	TGGTCCACTA	Gaggagg
	ACCATTAGACTCTC	ITTTATAGC	TCAACCCTTT	ACCAGGTGAT	CTCCTCC
551	CTATCTCAGCGCTT	TATTATTAC	AGTACTGGTG	GCACTCAGCT	TCCAACT
	GATAGAGTCGCGAA	ATAATAATG	TCATGACCAC	CGTGAGTCGA	AGGTTGA
601	CTGGCTCGTTCCTT	TATAATTTG	CATCCAAATG	ATTTCAGAAG	CAGCAAG
	GACCGAGCAAGGAA	ATATTAAAC	GTAGGTTTAC	TAAAGTCTTC	GTCGTTC
651		AGGGAGAAA	TGCGCACCAC	B D TTD CCTD C	
701	GATCTGCACCAGAT				

# FIGURE 46C (P2)

	CTAGACGTGGTCTAGGATCGCATTAATGTGAACTCTTATCAACCCCCTCT
751	CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAAT GAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
801	TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
851	TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
901	TCGTCACAGTTTTCTTTGCTTGGCATTGCTGTTCCTGGTAATTTTAATGC AGCAGTGTCAAAAGAAACGAACCGTAACGACAAGGACCATTAAAATTACG
951	TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
1001	GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTTGCGTTAT
1051	CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
1101	GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
1151	GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
1201	ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
1251	CAGATCTAGTCTAGTTTTAGCAGCGACATCAGGGAACAGTGGTACCACAC GTCTAGATCAGATC
1301	TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
1351	AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
1401	CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
1451	AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
1501	CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

## FIGURE 46C (P3)

#### GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACCACCATGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCGGTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP294

## FIGURE 46D

Amino acid sequence Comparison of Mutant Preproricin Linker region of Neutrophil elastase to Wild Type

Wild type ricin linker:

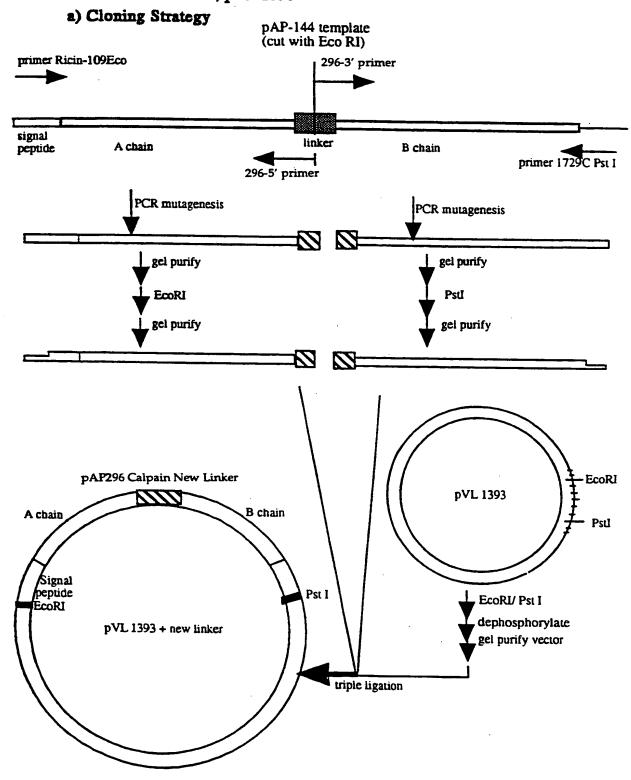
A chain- S L L I R P V V P N F N -B chain

pAP-294 (Neutrophil elastase) linker:

A chain- S L L G I A V P G N F N -B chain

#### FIGURE 47A

PCR Mutagenesis of Preprocicin Gene to Create A Variant Gene in Baculovirus Transfer Vector, pVL 1393



## FIGURE 47B

## Sequence of Calpain Linker Region

WT preprocin linker

primer 296-3' 5'- ACTCCTAGAACCCCCCCAGCTGATGTTTGT -3' *******
1) PCR mutagenesis
2) Ligate with pVL1393
pAP 296 linker (Calpain variant)
TITTTCAAAATATTGTT ACTCCTAGAACCCCCCCA

## FIGURE 47C (P1)

#### Sequence of pAP296 insert

	10	20	30	40	50
	l	i	t	ı	i
1	GAATTCATGAAACCG	GGAGGAAAT	ACTATTGTAP	TATGGATGTA'	でにしならず
	CTTAAGTACTTTGGC	CCTCCTTTA	TGATAACATT	יד בין ביים ביים ביים ביים ביים ביים ביים	ACCTCA
				.a.z.tuccinumii	ucg1 CM
51	GGCAACATGGCTTTG	<b>ヤヤヤイにこみ ヤ</b> ん	· ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・ ・	*************************	
	CCETTETACCENANC		JOHN LOOMS	FIGGICTITICA	CATTAG
	CCGTTGTACCGAAAC	MANCCIAG	GIGGAGTCCC	accagaaagt(	GTAATC
TO E	ACCETERCERCETE	TCCCC3 3 3 6			
101	AGGATAACAACATAT	TCCCCAAAC	AATACCCAAT	TATAAACTTT	ACCACA
	TCCTATTGTTGTATA	AGGGGTTTG	TTATGGGTT	latatttgaaa:	TGGTGT
7 5 7	CCCCCMCCCC cmcc				
131	GCGGGTGCCACTGTG	CAAAGCTAC	ACAAACTTT	<i><b>\TCAGAGCTGT'</b></i>	TCGCGG
	CGCCCACGGTGACAC	GTTTCGAT	TGTTTGAAAI	[AGTCTCGACA]	AGCGCC
201	TCGTTTAACAACTGG	agctgatgi	'GAGACATGA'	'ATACCAGTGT'	TGCCAA
	AGCAAATTGTTGACC	TCGACTACA	CTCTGTACTA	\TATGGTCACA	ACGGTT
251	ACAGAGTTGGTTTGC	CTATAAACC	AACGGTTTAT	TTTAGTTGAA	ביירים ב
	TGTCTCAACCAAACG	GATATTTGG	TTGCCAAATA	AAATCAACTT	CACACT
301	AATCATGCAGAGCTT	TCTGTTACA	TTACCCCTCC	ころでにでとれてとれる	MCC3.87
	TTAGTACGTCTCGAA	AGACAATGT	'AATCGCGAC	カナナロナ <i>でぴこでぴ</i> ぴ	IGCMIM
				.INCHGIGGII	ACGTAT
351	TGTGGTCGGCTACCG	<b>ጥርርጥርር እ</b> አ	<b>小力につこでかった。</b>		
	ACACCAGCCGATGGC	ACCACCTON	TAGCGCATA!	LITCTTTCATC	CTGACA
			AICGCGIATA	MAGAAAGTAG	GACTGT
401	ATCAGGAAGATGCAG	7 7 CC 7 7 CC 7	CEC3		
	ATCAGGAAGATGCAG		CTCATCTTT	CACTGATGTT	CAAAAT
	TAGTCCTTCTACGTC	TICGTIAGI	GAGTAGAAA	<b>AGTGACTACAA</b>	GTTTTA
451	CCATATACATTCCCC	**********************			
-02		TTTGGTGGT	AATTATGATI	AGACTTGAACA	ACTTGC
	GCTATATGTAAGCGG	AAACCACC	ITTAATACTA:	<b>ICTGAACTTGT</b>	TGAACG
501					
201		AAATATCG	GTTGGGAAA:	<b>IGGTCCACTAG</b>	AGGAGG
	ACCATTAGACTCTCT	TTTATAGCT	CAACCCTTT	ACCAGGTGATC	TCCTCC
E F 9					
551		ATTATTACE	GTACTGGTG	<b>SCACTCAGCTT</b>	ССААСТ
	GATAGAGTCGCGAAA	TAATAATGI	CATGACCAC	CGTGAGTCGAA	GGTTGA
601	CTGGCTCGTTCCTTT	ATAATTTG	ATCCAAATC	ATTTCDCDDCC	700770
	GACCGAGCAAGGAAA	TATTAAACO	TAGGTTTAC	TAAACTCTTCC	TOCHAG
651	ATTCCAATATATTGA TAAGGTTATATAACT	GGGAGAAAT	GCGCACCAC	4 A TTD C CTD C	B.C.C.
	TAAGGTTATATAACT	CCCTCTTT	CGCGTGCTC	・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・	ACCEGA
					IGGCCT
701	GATCTGCACCAGATC	CTAGCGTA	ים דידים בים בים בים	7 C 7 7 T 7 C 7 T 7 T	

## FIGURE 47C (P2)

- 751 CTTTCCACTGCAATTCAAGAGTCTAACCAAGGAGCCTTTGCTAGTCCAATGAAAGGTGACGTTAAGTTCTCAGATTGGTTCCTCGGAAACGATCAGGTTA
- 801 TCAACTGCAAAGACGTAATGGTTCCAAATTCAGTGTGTACGATGTGAGTA
  AGTTGACGTTTCTGCATTACCAAGGTTTAAGTCACACATGCTACACTCAT
- 851 TATTAATCCCTATCATAGCTCTCATGGTGTATAGATGCGCACCTCCACCA
  ATAATTAGGGATAGTATCGAGAGTACCACATATCTACGCGTGGAGGTGGT
- 951 TGATGTTTGTATGGATCCTGAGCCCATAGTGCGTATCGTAGGTCGAAATG ACTACAAACATACCTAGGACTCGGGTATCACGCATAGCATCCAGCTTTAC
- 1001 GTCTATGTGTTGATGTTAGGGATGGAAGATTCCACAACGGAAACGCAATA CAGATACACAACTACAATCCCTACCTTCTAAGGTGTTGCCTTTGCGTTAT
- 1051 CAGTTGTGGCCATGCAAGTCTAATACAGATGCAAATCAGCTCTGGACTTT GTCAACACCGGTACGTTCAGATTATGTCTACGTTTAGTCGAGACCTGAAA
- 1101 GAAAAGAGACAATACTATTCGATCTAATGGAAAGTGTTTAACTACTTACG CTTTTCTCTGTTATGATAAGCTAGATTACCTTTCACAAATTGATGAATGC
- 1151 GGTACAGTCCGGGAGTCTATGTGATGATCTATGATTGCAATACTGCTGCA CCATGTCAGGCCCTCAGATACACTACTAGATACTAACGTTATGACGACGT
- 1201 ACTGATGCCACCCGCTGGCAAATATGGGATAATGGAACCATCATAAATCC
  TGACTACGGTGGGCGACCGTTTATACCCTATTACCTTGGTAGTATTTAGG
- 1301 TTACAGTGCAAACCAACATTTATGCCGTTAGTCAAGGTTGGCTTCCTACT AATGTCACGTTTGGTTGTAAATACGGCAATCAGTTCCAACCGAAGGATGA
- 1351 AATAATACACAACCTTTTGTTACAACCATTGTTGGGCTATATGGTCTGTG
  TTATTATGTGTTGGAAAACAATGTTGGTAACAACCCGATATACCAGACAC
- 1401 CTTGCAAGCAAATAGTGGACAAGTATGGATAGAGGACTGTAGCAGTGAAA GAACGTTCGTTTATCACCTGTTCATACCTATCTCCTGACATCGTCACTTT
- 1451 AGGCTGAACAACAGTGGGCTCTTTATGCAGATGGTTCAATACGTCCTCAG
  TCCGACTTGTTGTCACCCGAGAAATACGTCTACCAAGTTATGCAGGAGTC
- 1501 CAAAACCGAGATAATTGCCTTACAAGTGATTCTAATATACGGGAAACAGT

#### FIGURE 47C (P3)

#### GTTTTGGCTCTATTAACGGAATGTTCACTAAGATTATATGCCCTTTGTCA

- 1601 TCAAGAATGATGGAACCATTTTAAATTTGTATAGTGGATTGGTGTTAGAT AGTTCTTACTACCTTGGTAAAATTTAAACATATCACCTAACCACAATCTA
- 1651 GTGAGGCGATCGGATCCGAGCCTTAAACAAATCATTCTTTACCCTCTCCA CACTCCGCTAGCCTAGGCTCGGAATTTGTTTAGTAAGAAATGGGAGAGGT

- 1801 GGACATTGTAAATTTTGTAACTGAAAGGACAGCAAGTTATATCGAATTCC CCTGTAACATTTAAAACATTGACTTTCCTGTCGTTCAATATAGCTTAAGG
- 1851 TGCAG ACGTC

Total number of bases is: 1855.

Sequence name: PAP296

## FIGURE 47D

Amino acid sequence Comparison of Mutant Preproricin Linker region of Calpain to Wild Type

Wild type ricin linker:

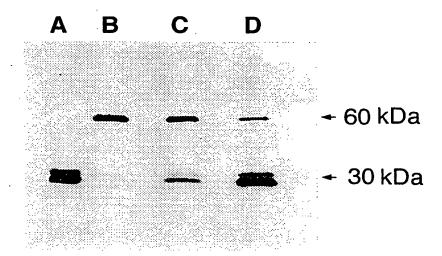
A chain-SLLIRPVVPNFN-B chain

pAP-296 (Calpain) linker:

A chain- FFKNIVTPRTPP-B chain

#### FIGURE 48

## Cleavage of pAP 214 by Cathepsin B



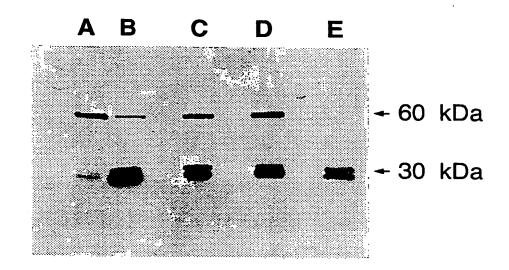
- A. Ricin standard
- B. pAP 214
- C. pAP 214 digested with 100 ng of Cathepsin B (18 hours)
- D. pAP 214 digested with 618 ng of Cathepsin B (18 hours)

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#### FIGURE 49

## Cleavage of pAP 220 with MMP-9

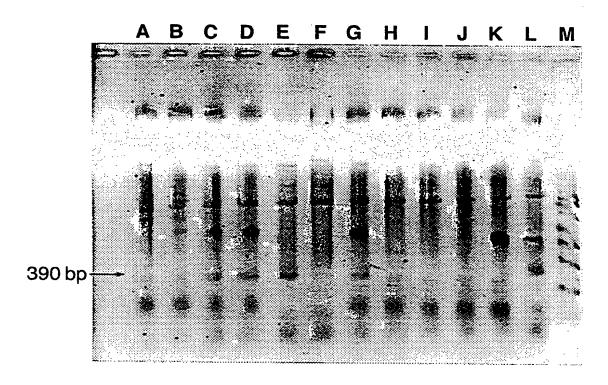


- **A.** pAP 220
- B. pAP 220 digested with 200 ng of MMP-9 (16 hrs)
- C. pAP 220 digested with 20 ng of MMP-9 (16hrs)
- D. pAP 220 digested with 20 ng of MMP-9 (2hrs)

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# FIGURE 50 Activation of pAP 214

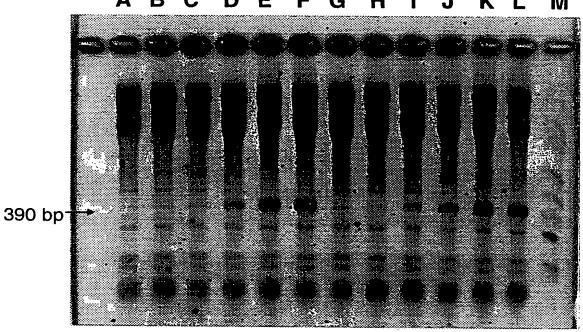


- A. 41.7 pg of pAP 214 digested with Cathepsin B
- B. 291 pg of pAP 214 digested with Cathpepsin B
- C. 2.0 ng of pAP 214 digested with Cathepsin B
- D. 14.2 ng of pAP 214 digested with Cathepsin B
- E. 100 ng of pAP 214 digested with Cathepsin B
- F. Negative control
- G. Ricin A chain
- H. 41.7 pg of pAP 214 variant
- J. 2.0 ng of pAP 214 variant
- K. 14.2 ng of pAP 214 variant
- L. 100ng of pAP 214 variant
- M. RNA ladder

#### FIGURE 51

## **Activation of pAP 220**

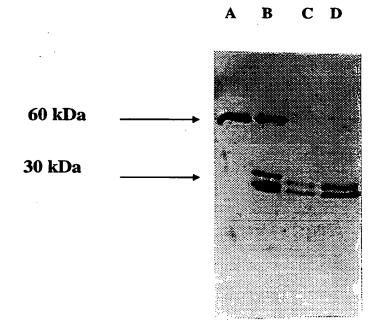
#### ABCDEFGHIJKLM



- A. 48.5 pg of pAP 220 variant
- B. 291 pg of pAP 220 variant
- C. 2.0 ng of pAP 220 variant
- **D.** 14.3 ng of pAP 220 variant
- E. 100 ng of pAP 220 variant
- F. Ricin A chain
- G. Negative Control
- H. 48.5 pg of pAP 220 variant digested with MMP-9
- I. 291 pg of pAP 220 variant digested with MMP-9
- J. 2.0 ng of pAP 220 variant digested with MMP-9
- K. 14.3 ng of pAP 220 variant digested with MMP-9
- L. 100 ng of pAP 220 variant digested with MMP-9
- M. RNA ladder

## FIGURE 52

Cleavage of pAP-248 Protein by The Human Cytomegalovirus (HCMV) protease

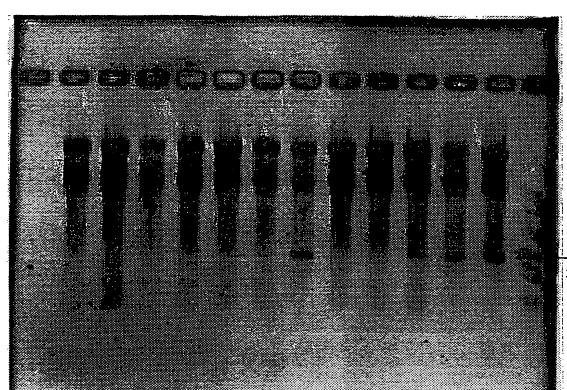


- A. pAP-248 (0.279 ug)
- B. pAP-248 protein (0.279 μg) digested with 0.25 μg of the HCMV protease
- C. Ricin standard (20 ng)
- D. Ricin standard (40 ng)

#### FIGURE 53

#### **Activation of pAP-248 Protein**

#### A B C D E F G H I J K L M

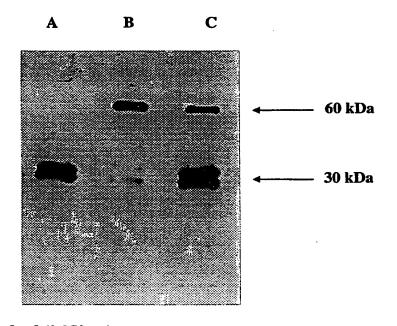


390 b fragment

- A. 90 ng of pAP-248 variant
- B. 12.8 ng of pAP-248 variant
- C. 1.8 ng of pAP-248 variant
- D. 260 pg pAP-248 variant
- E. 37 pg of pAP-248 variant
- F. Negative control
- G. Ricin A chain
- H. 37 pg of pAP-248 digested with HCMV protease
- I. 260 pg of pAP-248 digested with HCMV protease
- J. 1.8 ng of pAP-248 digested with HCMV protease
- K. 12.8 ng of pAP-248 digested with HCMV protease
- L. 90 ng of pAP-248 digested with HCMV protease
- M. RNA ladder

#### FIGURE 54

Cleavage of pAP-256 protein by The Hepatits A Virus 3C (HAV 3C) Protease

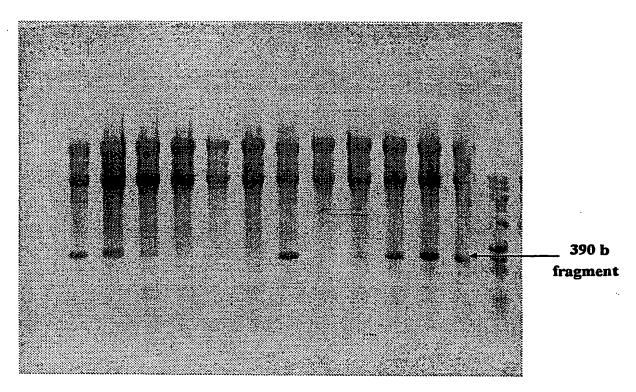


- A. Ricin standard (0.250 ug)
- B. pAP-256 protein (0.378 ug)
- C. pAP-256 protein digested (0.302 ug) with 1.25 µg of the HAV 3C protease

#### FIGURE 55

#### **Activation of pAP-256 Protein**

#### ABCDEFGHIJKLM



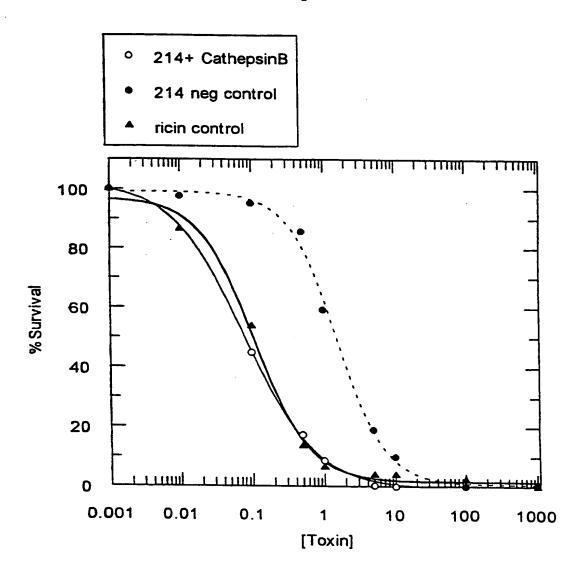
- A. 100 ng of pAP-256 variant
- B. 14.2 ng of pAP-256 variant
- C. 2.0 ng of pAP-256 variant
- D. 291 pg of pAP-256 variant
- E. 41.7 pg of pAP-256 variant
- F. Negative control
- G. Ricin A chain
- H. 41.7 pg of pAP-256 digested with HAV 3C protease
- I. 291 pg of pAP-256 digested with HAV 3C proteas
- J. 2.0 ng of pAP-256 digested with HAV 3C protease
- K. 14.2 ng of pAP-256 digested with HAV 3C protease
- L. 100 ng of pAP-256 digested with HAV 3C protease
- M. RNA ladder

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## FIGURE 56

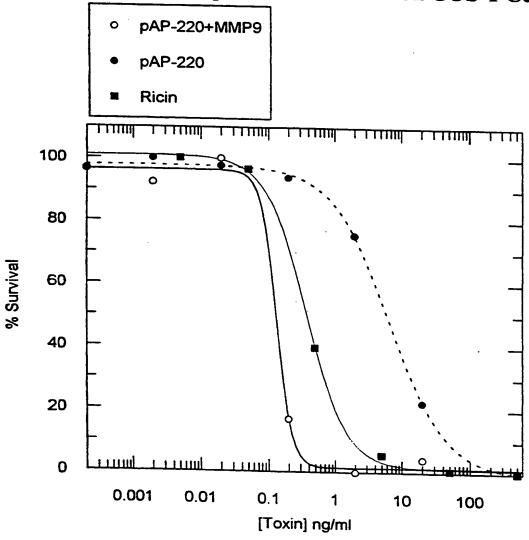
## Cytotoxicity of Digested and Undigested pAP 214 with Cathepsin B to COS-1 Cells



	Ricin	pAP 214	pAP 214 + Cathepsin B
IC <sub>50</sub> (ng/ml)	0.11	1.9	0.078
Relative Toxicity	1X	17X	0.7X

#### FIGURE 57

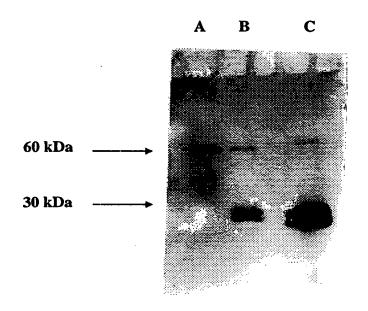
Cytotoxicity of pAP220 Digested with MMP-9 Compared to Freshly Thawed pAP220 and Ricin on COS-1 Cells



<u> </u>	Ricin	pAP 220	pAP 220 + MMP-9
IC <sub>50</sub> (ng/ml)	0.31	6.7	0.12
Relative Toxicity	IX	22X	0.13
		227	0.4X

## FIGURE 58

#### Cleavage of pAP-270 protein by The Matrix Metalloproteinase 2 (MMP-2)

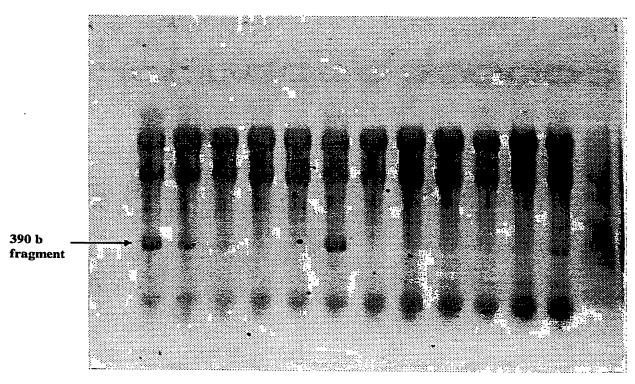


- A. pAP-270 (0.120  $\mu$ g) undigested
- B. pAP-270 (0.120  $\mu g)$  digested with 0.250  $\mu g$  MMP-2
- C. Ricin Standard (0.05 µg)

#### FIGURE 59

#### Activation of pAP-270 protein

#### ABCDEFGHIJKLM

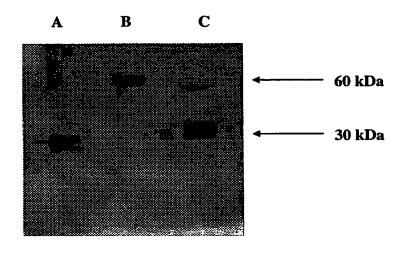


- A. 100 ng of digested pAP-270
- B. 14.2 ng of digested pAP-270
- C. 2.0 ng of digested pAP-270
- D. 290 pg of digested pAP-270
- E. 46 ng of digested pAP-270
- F. Ricin A chain
- G. Negative control
- H. 46 pg of pAP-270
- I. 290 pg of pAP-270
- J. 2.0 ng of pAP-270
- K. 14.2 ng of pAP-270
- L. 100 ng of pAP-270

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#### FIGURE 60

#### Cleavage of pAP-288 protein by Plasminogen Tissue Activator (t-PA)

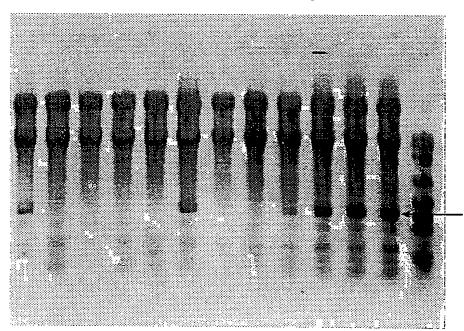


- A. Ricin Standard (0.05µg)
- B. pAP-288 (0.66  $\mu$ g) undigested
- C. pAP-288 (0.60  $\mu g$ ) digested with 0.18  $\mu g$  of t-PA protease

#### FIGURE 61

#### Activation of pAP-288 protein

#### A B C D E F G H I J K L M

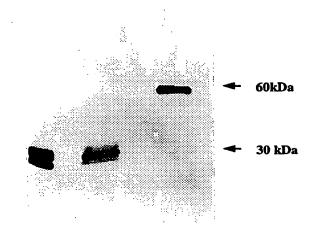


390 b fragment

- A. 200 ng of pAP-288
- B. 28.4 ng of pAP-288
- C. 4.0 ng of pAP-288
- D. 482 pg of pAP-288
- E. 83.4 pg of pAP-288
- F. Ricin A chain
- G. Negative control
- H. 83.4 pg of pAP-288 digested with tissue Plasminogen Activator (t-PA)
- I. 482 pg of pAP-288 digested with t-PA
- J. 4.0 ng of pAP-288 digested with t-PA
- K. 28.4 ng f pAP-288 digested with t-PA
- L. 200 ng of pAP-288 digested with t-PA
- M. RNA ladder

#### FIGURE 62

#### Cleavage of pAP 294 With Human Neutrophil Elastase



- A. Ricin Standard ( 0.050 μg)
- B. pAP 294 protein (  $0.171\ \mu g)$  digested with 1.42  $\mu g$  of Human Neutrophil Elastase
- C. pAP 294 protein ( $0.121 \mu g$ )

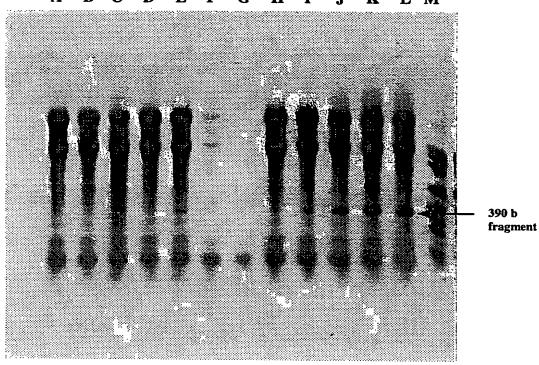
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#### FIGURE 63

#### **Activation of pAP 294 Protein**

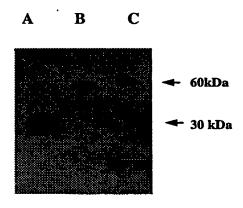
#### A B C D E F G H I J K L M



- A. 60 ng of pAP 294
- B. 8..57 ng of pAP 294
- C. 1.22 ng of pAP 294
- D. 175 pg of pAP 294
- E. 25 pg of pAP 294
- F. Ricin A chain
- **G.** Negative Control
- H. 360 ng of pAP 294 digested with Human Neutrophil Elastase
- I. 51 ng of pAP 294 digested with Human Neutrophil Elastase
- J. 7.3 ng of pAP 294 digested with Human Neutrophil Elastase
- K. 1.0 ng of pAP 294 digested with Human Neutrophil Elastase
- L. 150 pg of pAP 294 digested with Human Neutrophil Elastase
- M. RNA ladder

## FIGURE 64

#### Cleavage of pAP 296 with Calpain

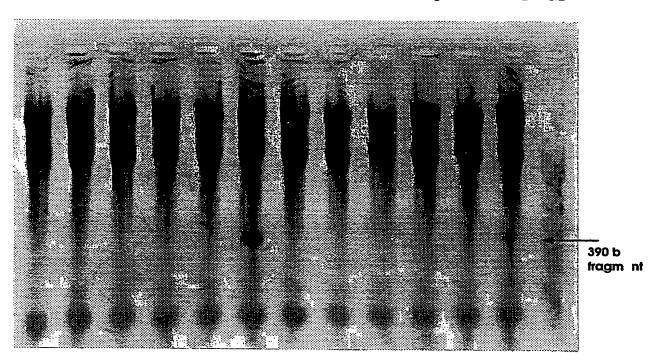


- A. Ricin Standard (0.05 μg)
- B. pAP 296 (0.761  $\mu g$ ) undigested
- C. pAP 296 (0.761  $\mu g$  ) digested with 4.0  $\mu g$  of Calpain

#### FIGURE 65

#### **Activation of pAP 296 Protein**

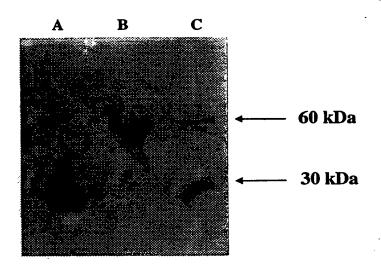
#### A B C D E F G H I J K L M



- A. 100 ng of pAP 296 variant
- B. 14.2 ng of pAP 296 variant
- C. 2.0 ng of pAP 296 variant
- D. 290 pg of pAP 296 variant
- E. 46 pg of pAP 296 variant
- F. Ricin A chain
- G. Negative control
- H. 46 pg of pAP 296 variant digested with Calpain
- I. 290 pg of pAP 296 variant digested with Calpain
- J. 2.0 ng of pAP 296 variant digested with Calpain
- K. 14.2 ng of pAP 296 variant digested with Calpain
- L. 100 ng of pAP 296 variant digested with Calpain
- M. RNA ladder

## FIGURE 66

Cleavage of pAP-222 Protein by The Matrix Metalloproteinase 2 (MMP-2)

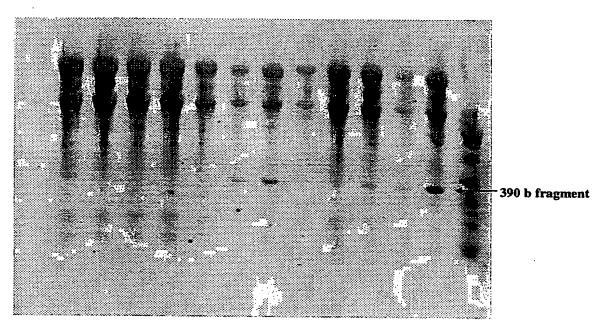


- A. Ricin Standard (0.250 ug)
- B. pAP-222 Protein (0.250 ug)
- C. pAP-222 protein (0.250 ug) digested with 0.28 ug of MMP-2

#### FIGURE 67

#### Activation of pAP-222 Protein

#### A B C D E F G H I J K L M



- A. 100 ng of pAP-222 variant
- **B.** 14.2 ng of pAP-222 variant
- C. 2.0 ng of pAP-222 variant
- D. 291 pg of pAP-222 variant
- E. 41.7 pg of pAP-222 variant
- F. Ricin A chain
- G. Ricin A chain
- H. 41.7 pg of pAP-222 digested with MMP-2
- I. 291 pg of pAP-222 digested with MMP-2
- J. 2.0 ng of pAP-222 digested with MMP-2
- K. 14.2 ng of pAP-222 digested with MMP-2
- L. 100 ng of pAP-222 digested with MMP-2
- M. RNA ladder

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#### INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6:

C12N 15/29, 15/62, 15/70, 15/86, A61K 38/16, 48/00

(11) International Publication Number:

WO 98/49311

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(43) International Publication Date:

5 November 1998 (05.11.98)

(21) International Application Number:

PCT/CA98/00394

A<sub>3</sub>

(22) International Filing Date:

30 April 1998 (30.04.98)

(30) Priority Data:

60/045,148 60/063,715 30 April 1997 (30.04.97)

29 October 1997 (29.10.97)

US US

(71) Applicant (for all designated States except US): DE NOVO ENZYME CORPORATION [CA/CA]; #2 Suite SFU Discovery Park, Burnaby, British Columbia V5A 1S6 (CA).

(72) Inventor; and

(75) Inventor/Applicant (for US only): BORGFORD, Thor [CA/CA]; 443 Fadar Street, New Westminster, British Columbia V3L 3T2 (CA).

(74) Agent: BERESKIN & PARR; 40th floor, 40 King Street West, Toronto, Ontario M5H 3Y2 (CA). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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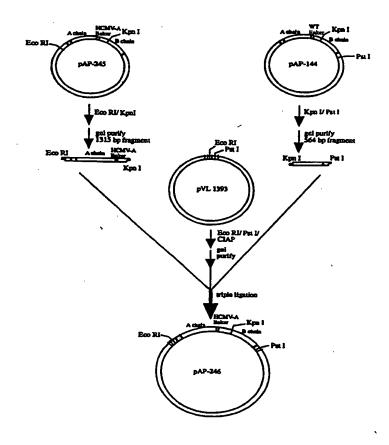
(88) Date of publication of the international search report:

11 February 1999 (11.02.99)

(54) Title: RICIN-LIKE TOXIN VARIANTS FOR TREATMENT OF CANCER, VIRAL OR PARASITIC INFECTIONS

#### (57) Abstract

The present invention provides a protein having an A chain of a ricin-like toxin, a B chain of a ricin-like toxin and a heterologous linker amino acid sequence, linking the A and B chains. The linker sequence contains a cleavage recognition site for a disease specific protease such as a cancer, fungal, viral or parasitic protease. The invention also relates to a nucleic acid molecule encoding the protein and to expression vectors incorporating the nucleic acid molecule. Also provided is a method of inhibiting or destroying mammalian cancer cells, cells infected with a virus, a fungus, or parasite, or parasites utilizing the nucleic acid molecules and proteins of the invention and pharmaceutical compositions for treating human cancer, viral infection, fungal infection, or parasitic infection.



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#### INTERNATIONAL SEARCH REPORT

ional Application No Into PCT/CA 98/00394

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/62 A61K38/16 C12N15/70 C12N15/86 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

#### B. FIELDS SEARCHED

 $\begin{array}{ll} \text{Minimum documentation searched (classification system followed by classification symbols)} \\ IPC~6~~CO7K~~C12N~~A61K \end{array}$ 

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

	•	- · · · · · · · · · · · · · · · · · · ·
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 18332 A (US HEALTH) 18 August 1994	1-10, 12-23, 25-35
	* see the whole document, esp. p.16 1.30 - p.20 1.2 *	
Y	WESTBY M ET AL: "PREPARATION AND CHARACTERIZATION OF RECOMBINANT PRORICIN CONTAININGAN ALTERNATIVE PROTEASE-SENSITIVE LINKER SEQUENCE" BIOCONJUGATE CHEMISTRY, vol. 3, no. 5, 1 January 1992, pages 375-381, XP000578216 cited in the application * see the whole document, esp. last paragraph *	1-10, 12-23, 25-35

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Date of the actual completion of theinternational search  27 October 1998	Date of mailing of the international search report $10/11/1998$
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#### INTERNATIONAL SEARCH REPORT

It ational Application No
PCT/CA 98/00394

C.(Continu	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	LEPPLA S H ET AL: "DEVELOPMENT OF ANTHRAX-TOXIN BASED FUSION PROTEINS FOR TARGETING OFHIV-1-INFECTED CELLS" ZENTRALBLATT FUER BAKTERIOLOGIE. SUPPLEMENT, vol. 24, 1994, pages 431-442, XP002041056 cited in the application * see the whole document, esp. pp.437-39 *	1-35
A	COOK J P ET AL: "BIOLOGICALLY ACTIVE INTERLEUKIN 2-RICIN A CHAIN FUSION PROTEINS MAYREQUIRE INTRACELLULAR PROTEOLYTIC CLEAVAGE TO EXHIBIT A CYTOTOXIC EFFECT". BIOCONJUGATE CHEMISTRY, vol. 4, no. 6, 1 November 1993, pages 440-447, XP000417282 see the whole document	1-35
A	O'HARE M ET AL: "CYTOTOXICITY OF A RECOMBINANT RICIN-A-CHAIN FUSION PROTEIN CONTAINING A PROTEOLYTICALLY-CLEAVABLE SPACER SEQUENCE" FEBS LETTERS, vol. 273, no. 1/02, 29 October 1990, pages 200-204, XP002041057 cited in the application see the whole document	1-35
<b>A</b> _	PANCHAL R. ET AL.: "Tumor protease-activated, pore-forming toxins from a combinatorial library" NATURE BIOTECHNOLOGY, vol. 14, no. 7, 14 July 1996, pages 852-856, XP002082096 cited in the application see the whole document	1-35
A	EP 0 466 222 A (DOWELANCO) 15 January 1992 cited in the application see the whole document	1-35
Ρ,Χ	WO 97 41233 A (NOVO ENZYME CORP DE; BORGFORD THOR (CA)) 6 November 1997  see the whole document	1-10, 12-23, 25-35

ernational application No.

#### INTERNATIONAL SEARCH REPORT

PCT/CA 98/00394

Box I Observations whire certain claims were found unsearchable (Continuation of item 1 of first shiet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: Remark: Although claims 25-30, 32, 33 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

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Information on patent family members

In: :tional Application No
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Patent document cited in search report		Publication date .	Patent family member(s)		Publication date	
WO 9418332	A	18-08-1994	US	5591631 A	07-01-1997	
			US	5677274 A	14-10-1997	
			AT	169959 T	15-09-1998	
			AU	682500 B	09-10-1997	
•			AU	6392294 A	29-08-1994	
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